ATM physically and functionally interacts with PCNA to regulate DNA synthesis

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Acknowledgements

Jason White, PhD

2006-2009

Serah Choi, PhD
(MSTP Student)

2007-2011

Armin Gamper, PhD

2010-present

Katherine Fu

2011-present

Sections

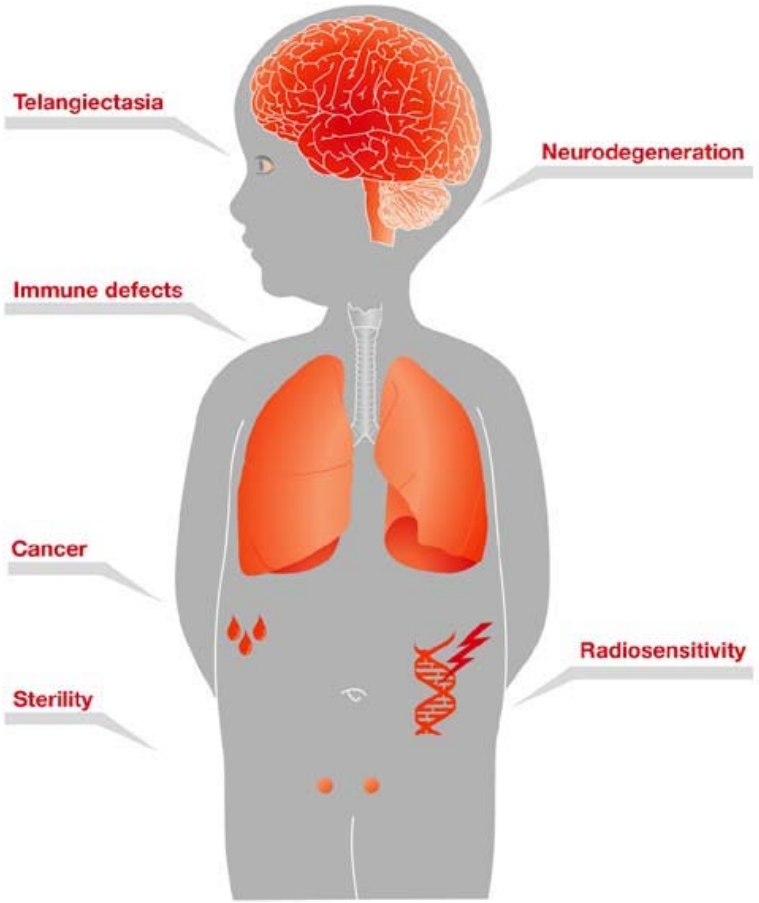
1. **Irreversible damage** accumulates rapidly when ATM kinase activity is inhibited in irradiated cells

2. **Chemically-inhibited ATM kinase blocks DNA repair** in a manner that does not occur in the absence of ATM protein

3. **ATM interacts with PCNA** *in vivo* and *in vitro*

4. A 20 amino acid **ATM peptide stimulates DNA synthesis** by DNA polymerase δ *in vitro*

Ataxia telangiectasia (A-T)

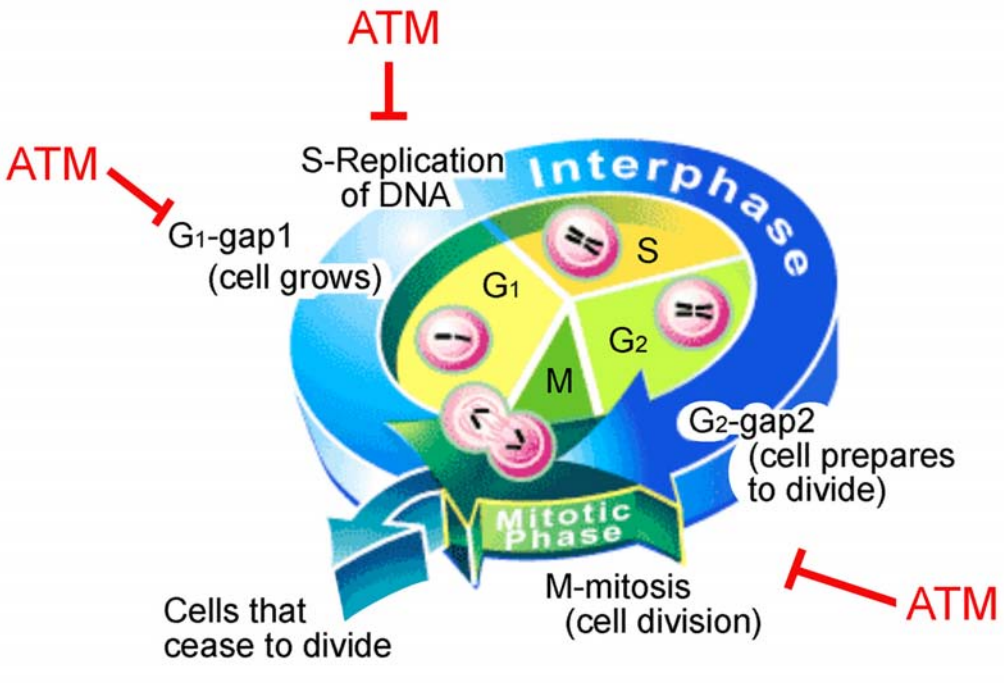


- **Rare, autosomal recessive disorder**
  - Progressive neurodegeneration
  - Immunodeficiency
  - Increased risk of lymphoid malignancies
  - Sterility
  - Interstitial lung disease
  - ***Profound radiosensitivity***

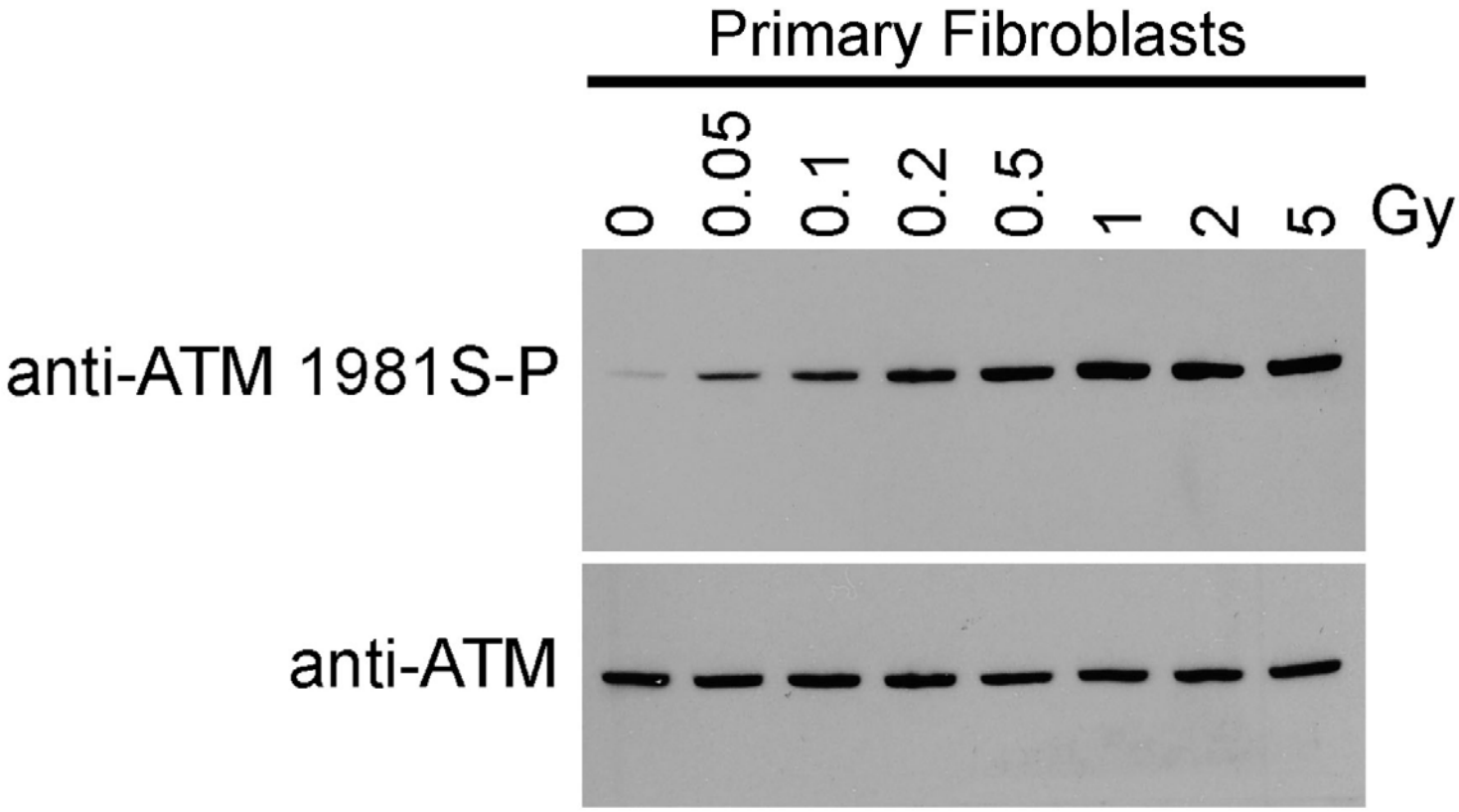
McKinnon. EMBO reports ***5****, 8, 772-776 (2004)*

Cells derived from A-T individuals

- **Checkpoint defects**
  - Diminished p53-mediated G_1 arrest
  - Radioresistant DNA synthesis (RDS)
  - Attenuated G_2 arrest
- **Exhibit chromosomal instability**
- ***Hypersensitive to IR***



ATM kinase activation can be detected within 15 min following 0.05 Gy IR



Bakkenist CJ, Kastan MB (2003) Nature 421, 499-506
White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

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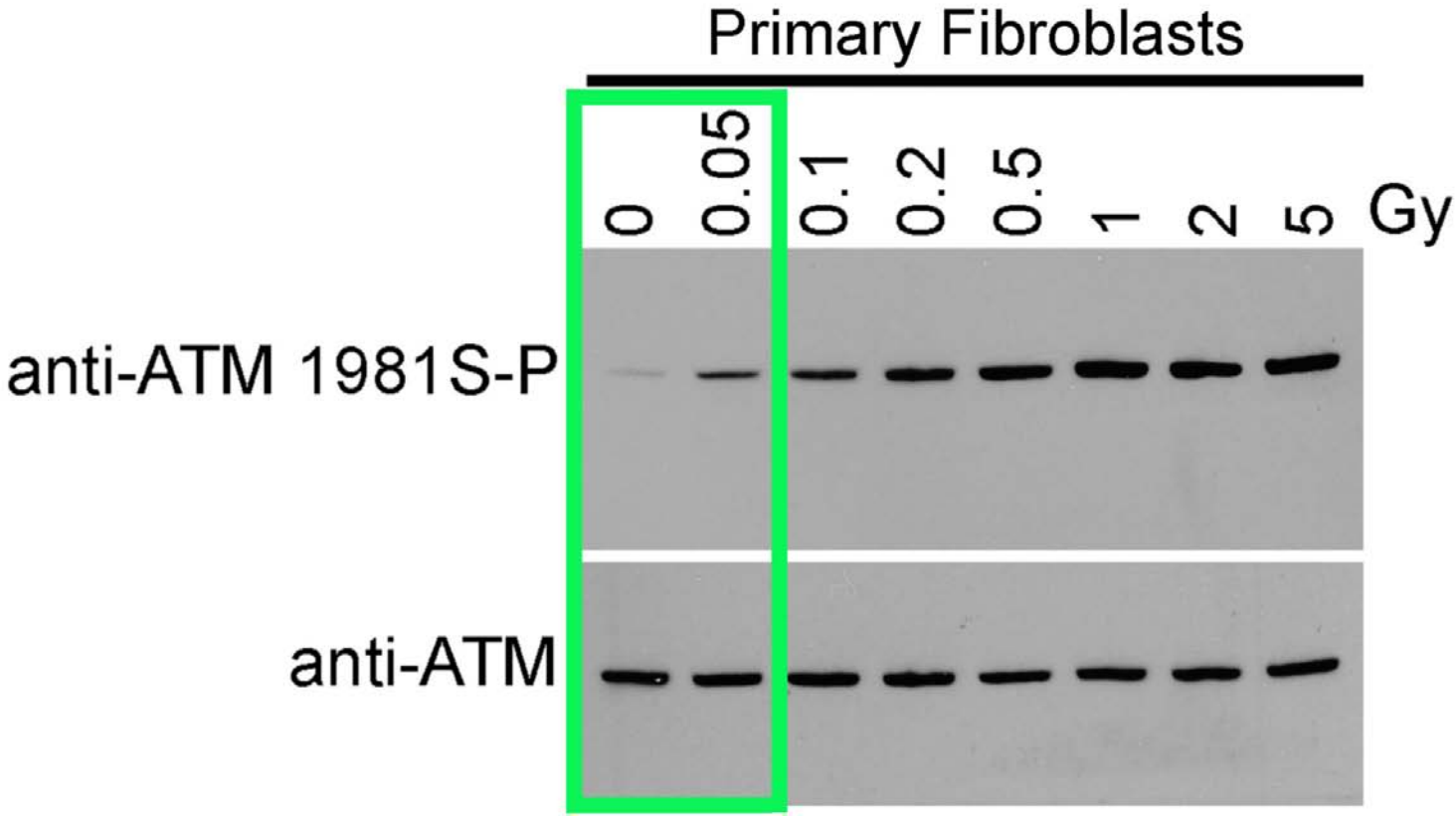

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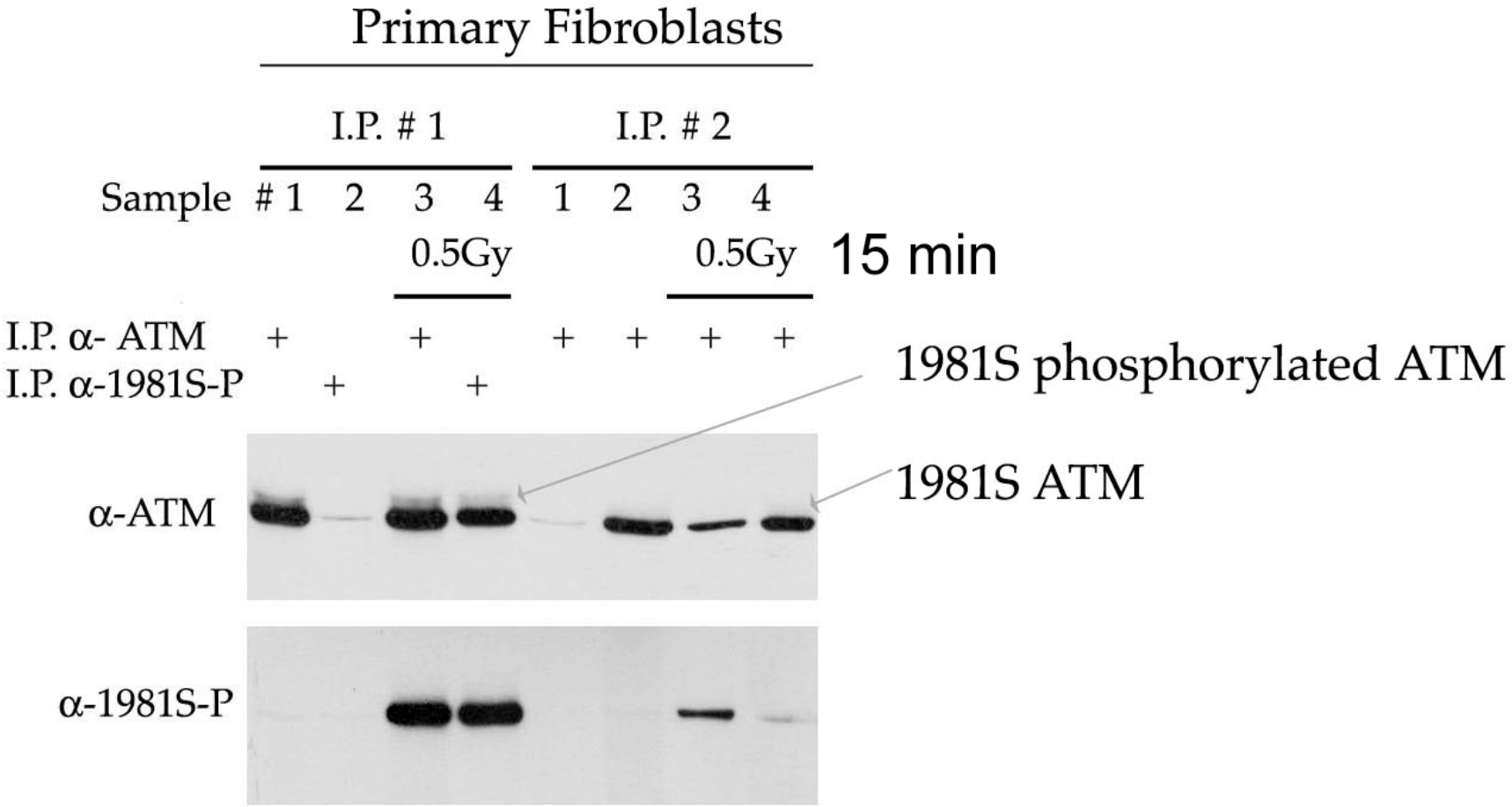
Over 50% of ATM kinase is phosphorylated within 15 min following 0.5 Gy IR



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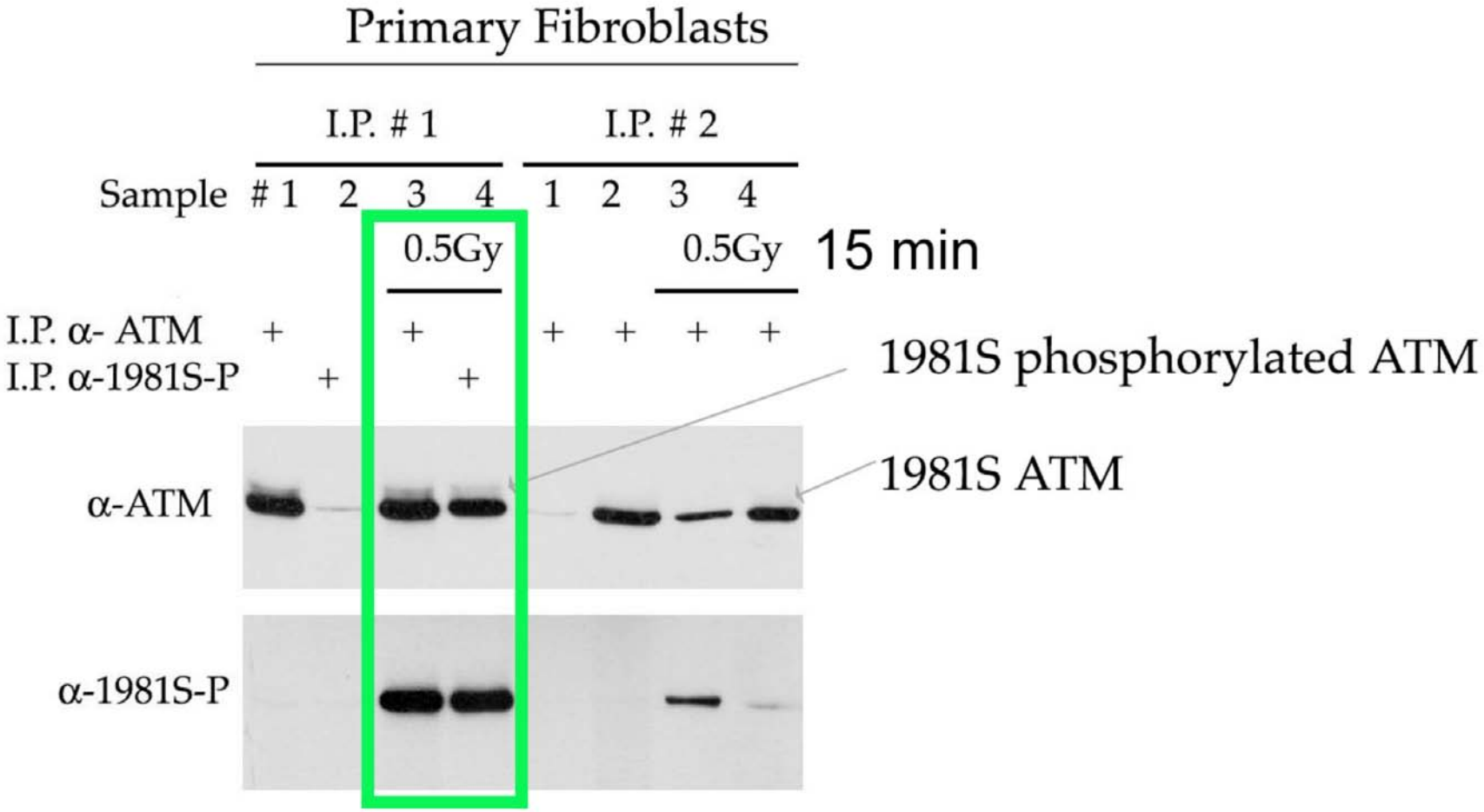
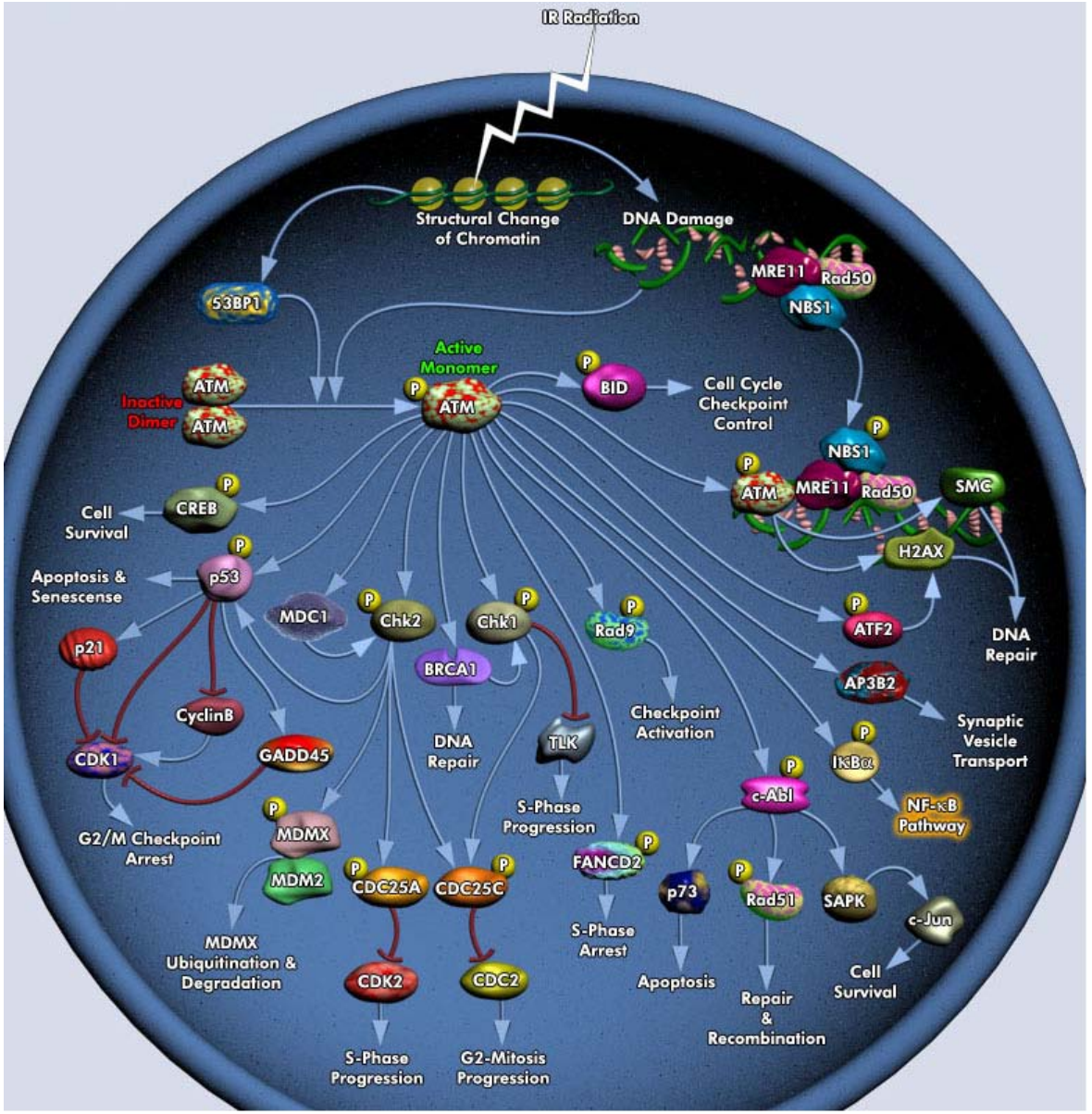


Bakkenist CJ, Kastan MB (2003) Nature 421, 499-506

ATM encodes a kinase

- **ATR is a related kinase with very similar substrate specificity**
- **>700 substrates identified**
  - Matsuoka S et al. (2007) Science 316, 1160-1166
- **>1000 substrates**
  - Olsen JV et al. (2010) Science Signaling 3(104):ra3
- Bensimon A et al. (2010)Science Signaling 3(151):rs3
- Serah Choi et al. in preparation



~60 percent of people with cancer are treated with radiation therapy

Breast cancer is dosed at 50 Gy in fractions of 1.8 to 2 Gy with an additional 10 Gy boost to the surgical bed site

Pancreatic cancer is dosed at 50.4 Gy in fractions of 1.8 Gy

Lung cancer is dosed at 45 Gy to 66 Gy in fractions of 1.8 Gy to 2.1 Gy

Goal

In order to identify essential ATM kinase-dependent signaling within this system new tools are needed to dissect the response

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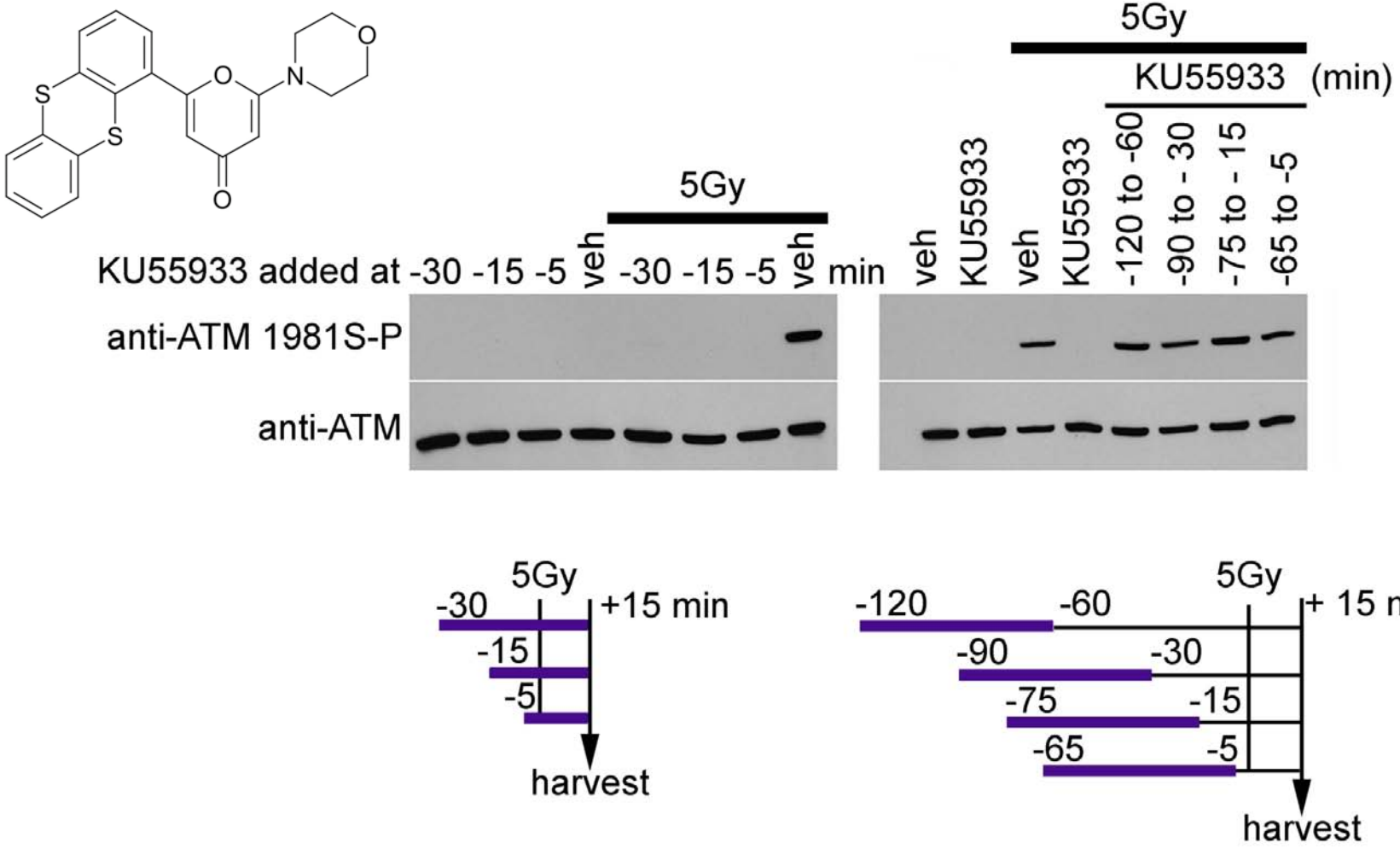
KU55933 can be used as a "molecular switch" to temporally dissociate ATM kinase signaling in cells



White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-84

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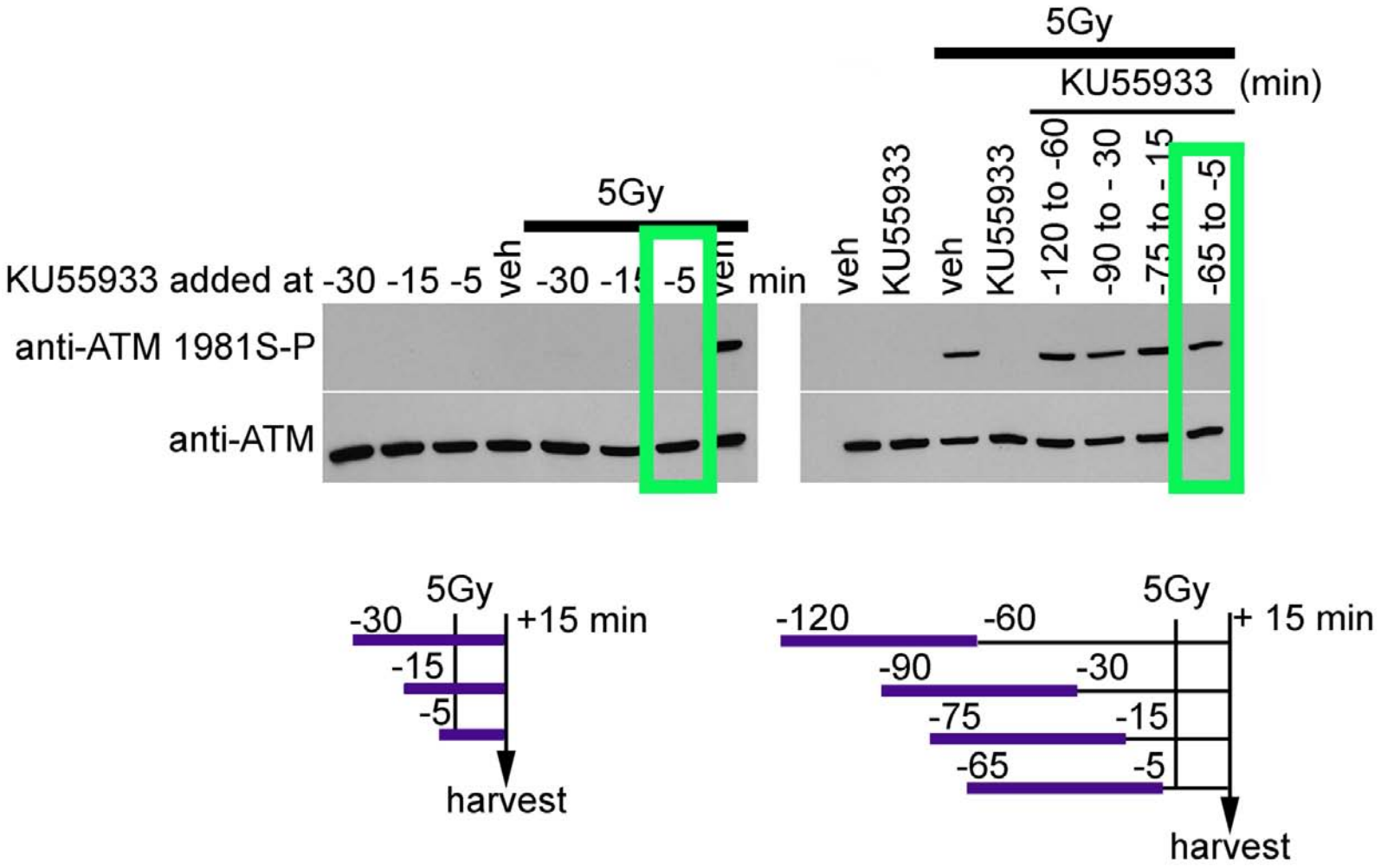
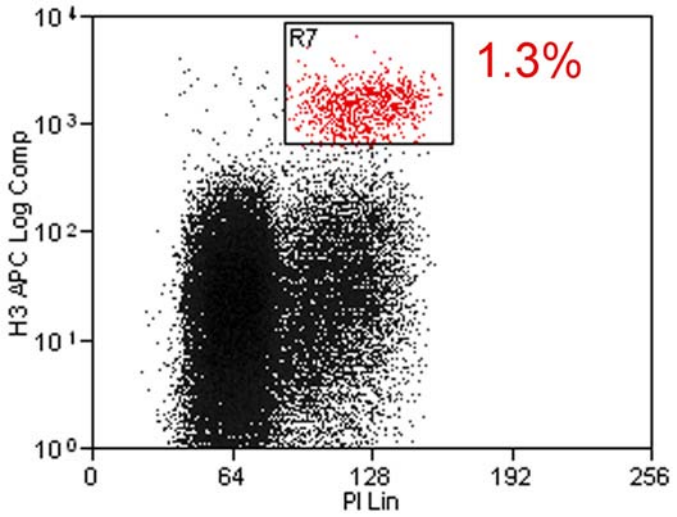


White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-84

G_2/M cell cycle checkpoint assay

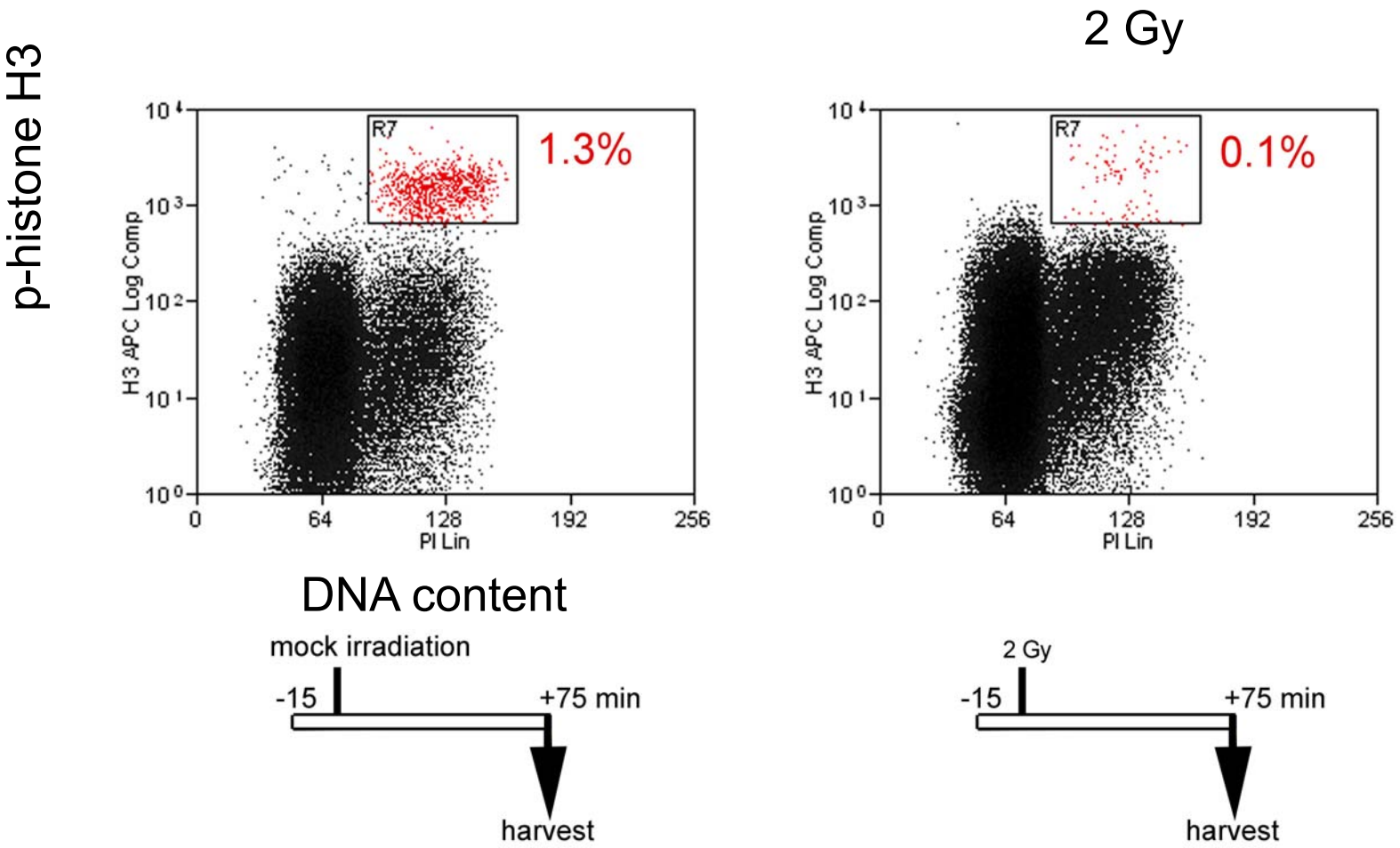
p-histone H3

IMR90

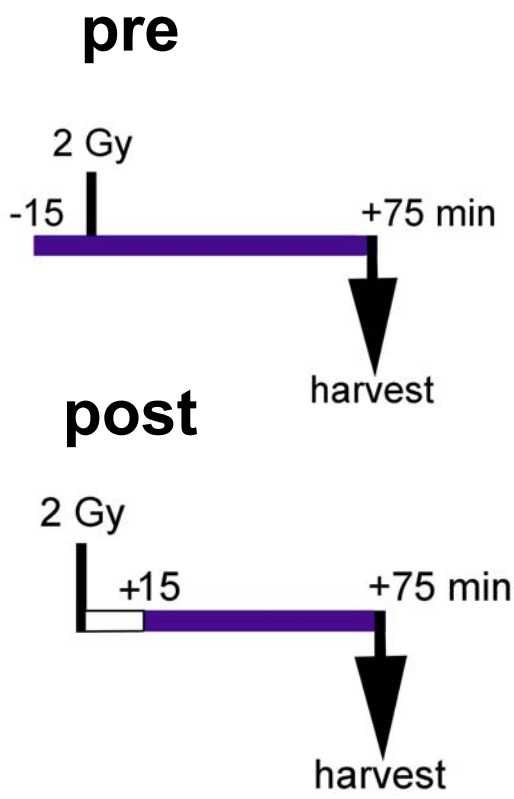


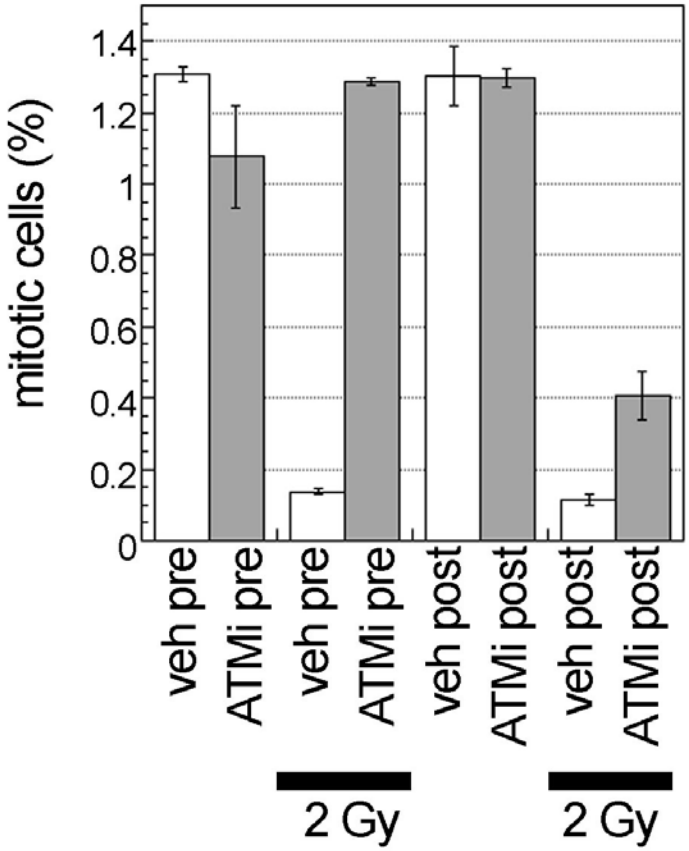
DNA content

G_2/M cell cycle checkpoint assay



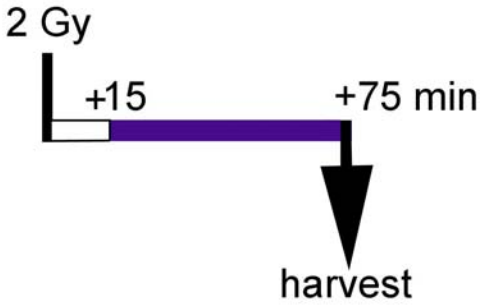
ATM kinase activity from 0 to +15 min following IR is sufficient to activate the G_2/M cell cycle checkpoint

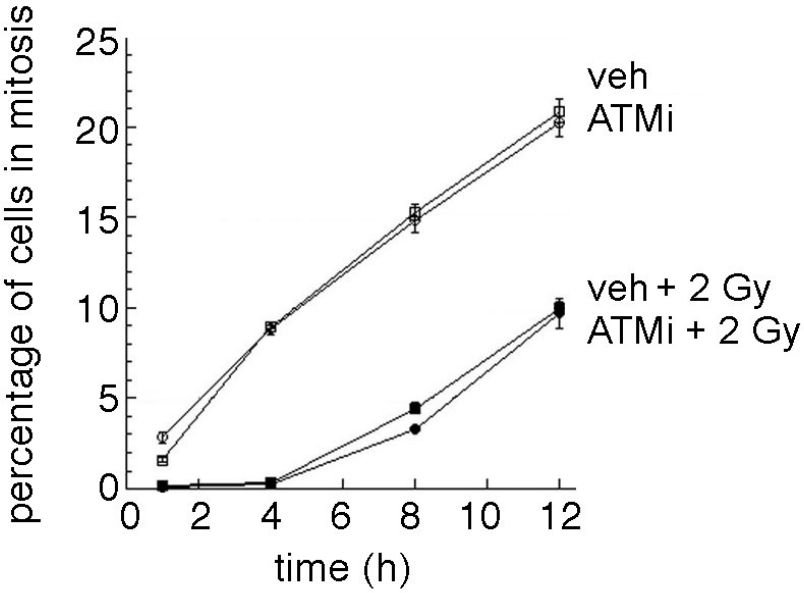




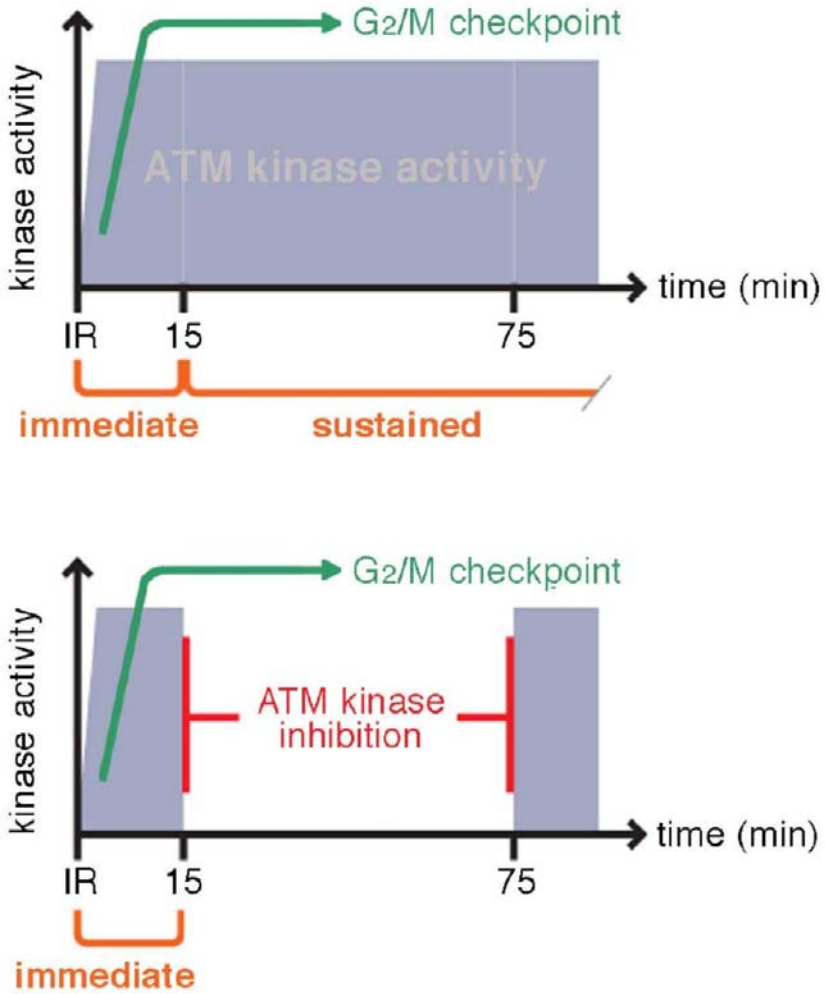
White JS, Choi S, Bakkenist CJ. (2010). Science Signaling 3(124):ra44

G_2/M cell cycle checkpoint recovery is not compromised by transient ATM kinase inhibition

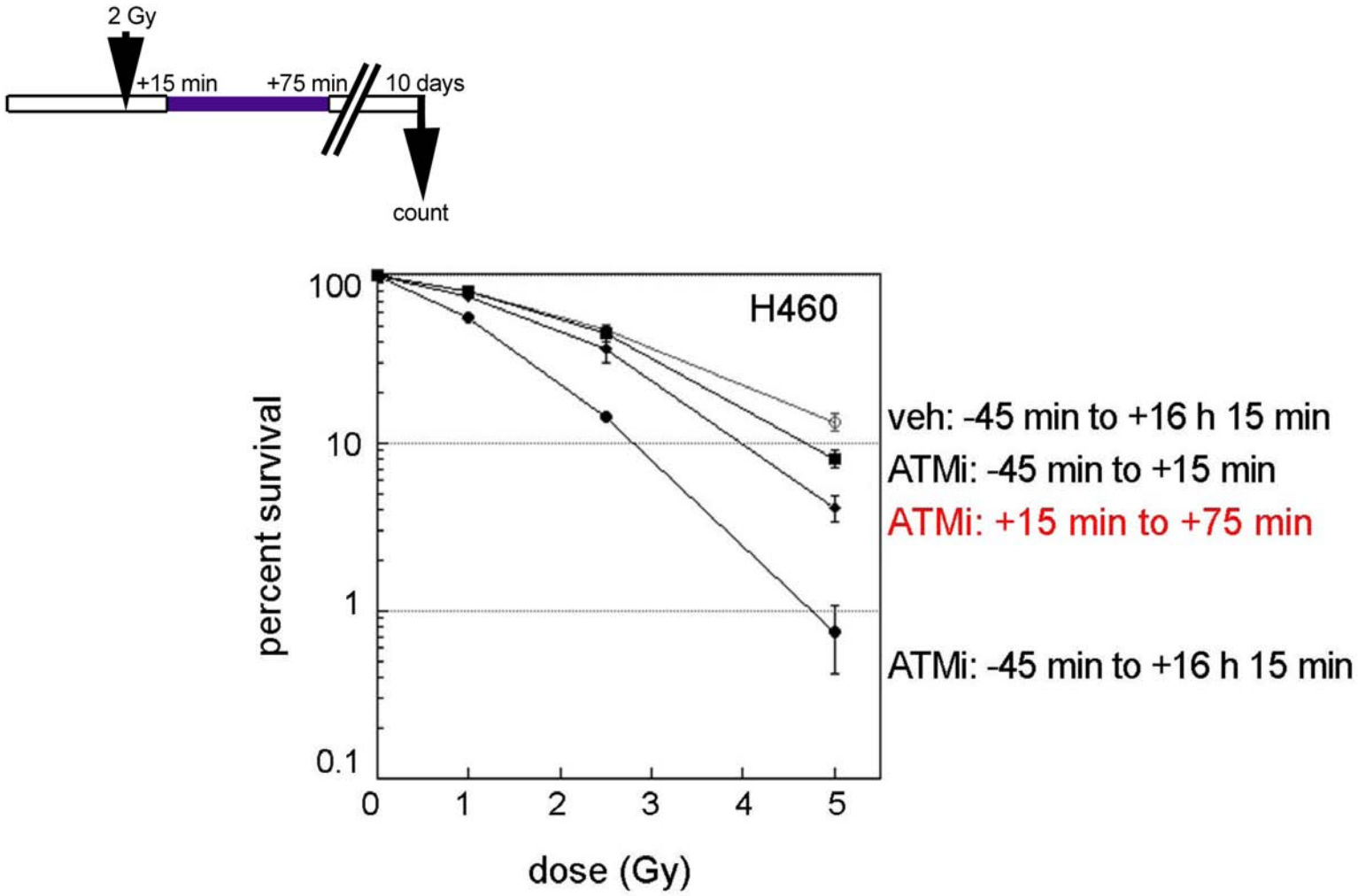




The G_2/M cell cycle checkpoint is intact when ATM kinase is inhibited from +15 to +75 min following IR



Transient inhibition of ATM kinase activity is sufficient to radiosensitize cells



White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

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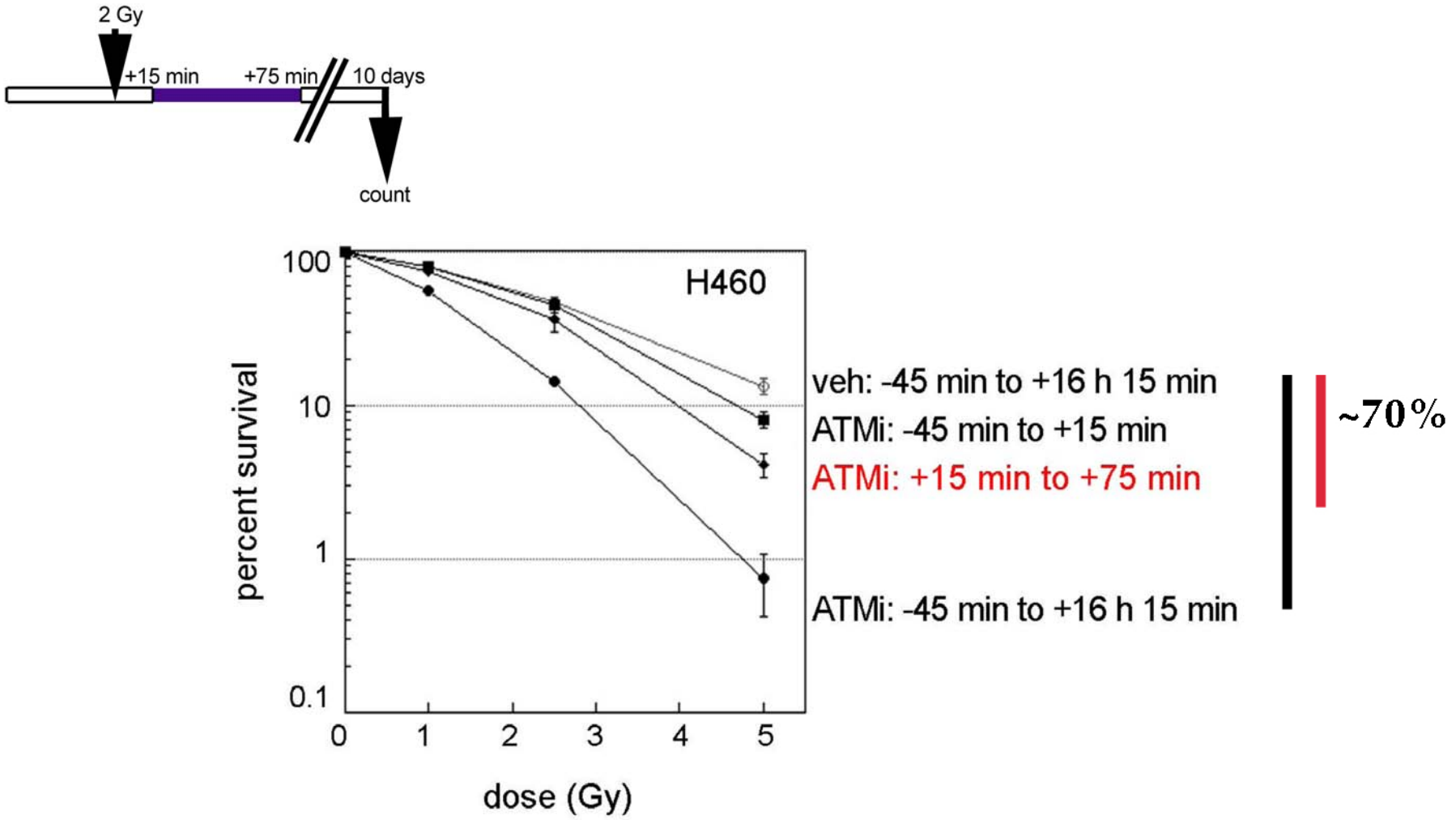


White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

Chromosome breakage assay

Cells were harvested 48 h following irradiation using 2 methods:

Colcemid

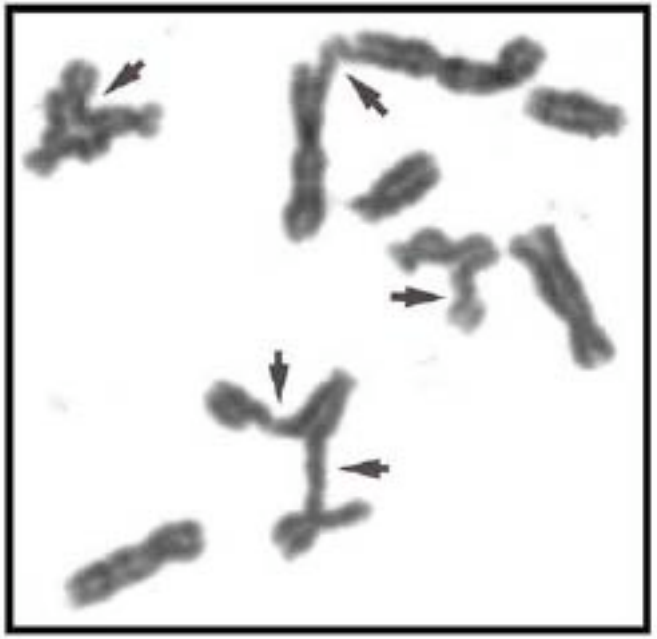
microtubule inhibitor

M-phase cells

Calyculin A

PP1 and PP2A inhibitor that prematurely condenses chromatin

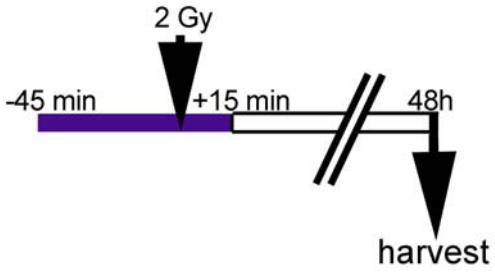
late S- and G_2-phase cells



50 “blinded” spreads were scored for each treatment

Transient ATM kinase inhibition from +15 min to +75 min accumulates persistent chromatid aberrations in late-S and G_2, but not M phase cells

pre



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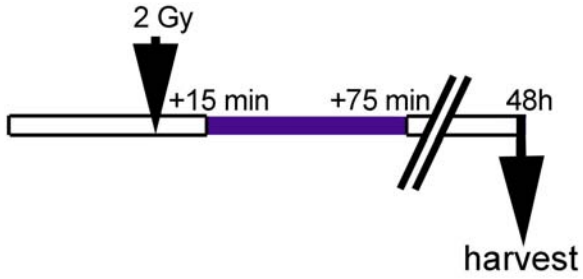

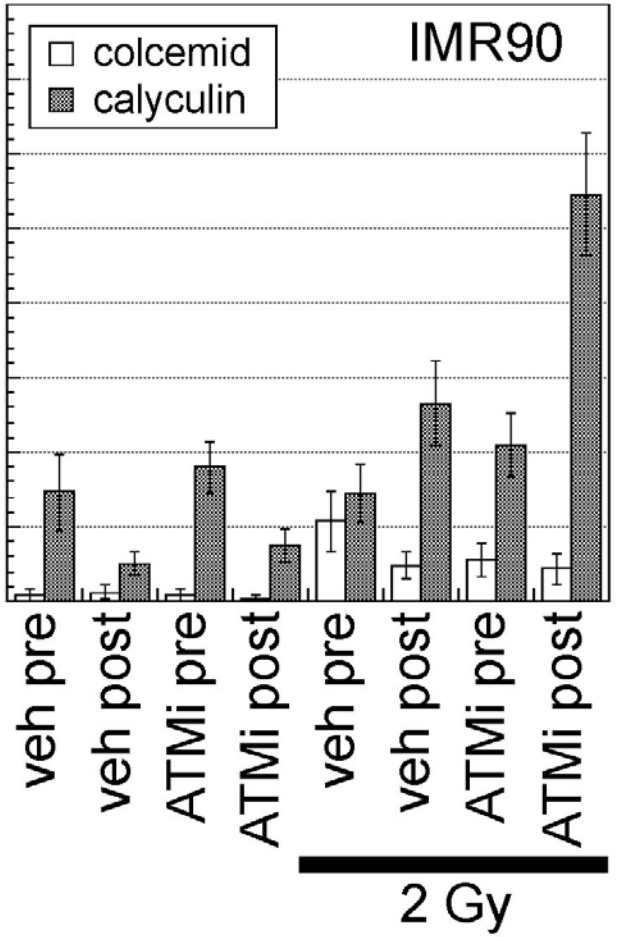

White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

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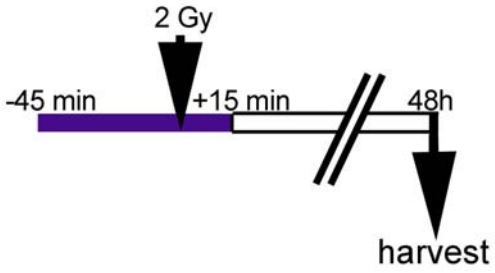

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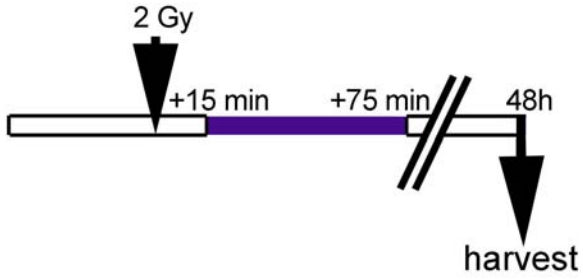
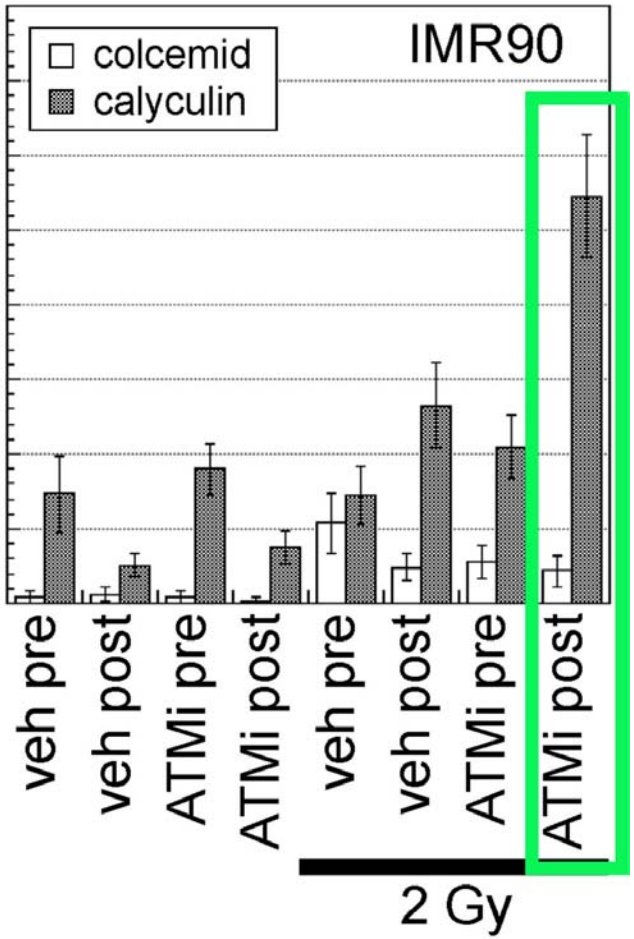




White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

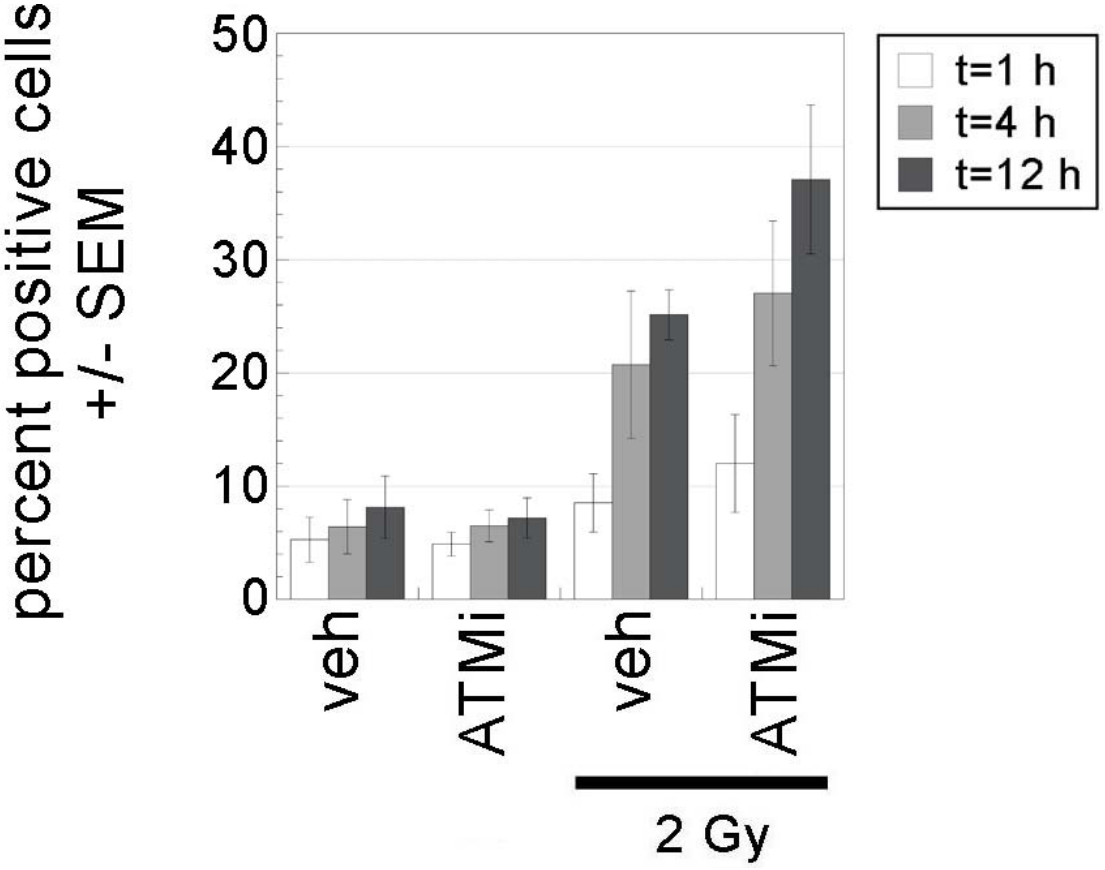
Summary Section 1

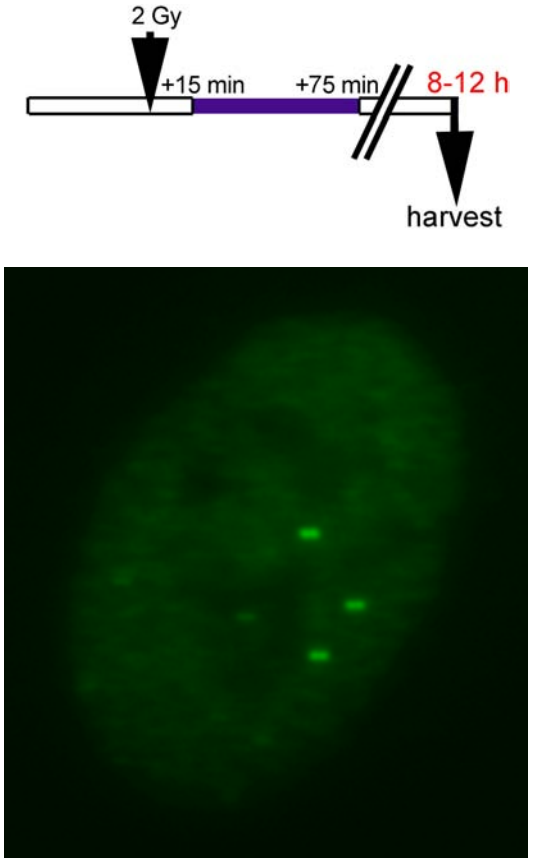
The cellular radiosensitization observed when ATM kinase is inhibited cannot be attributed to abrogation of the G_2/M cell cycle checkpoint

Irreversible damage accumulates rapidly when ATM kinase activity is inhibited in irradiated cells

Persistent chromatid breaks accumulate in *late-S- and G_2-phase* cells when ATM kinase activity is inhibited

RPA foci accumulate at 12 h when ATM kinase is inhibited from +15 min to + 75 min following IR

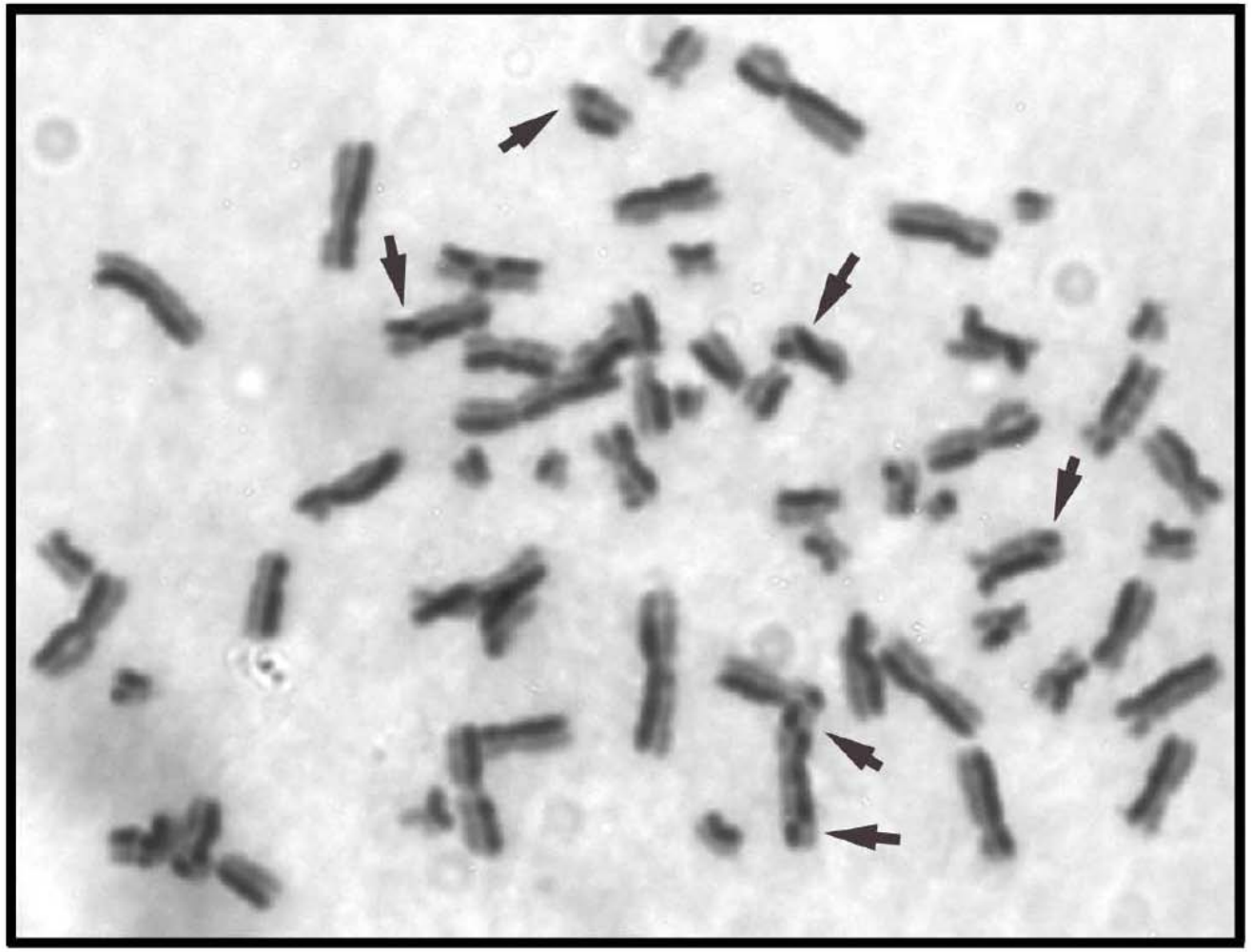




2 Gy + KU55933, t = 12 h

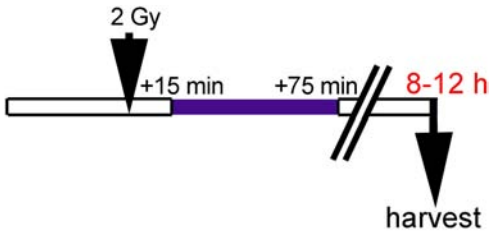
White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

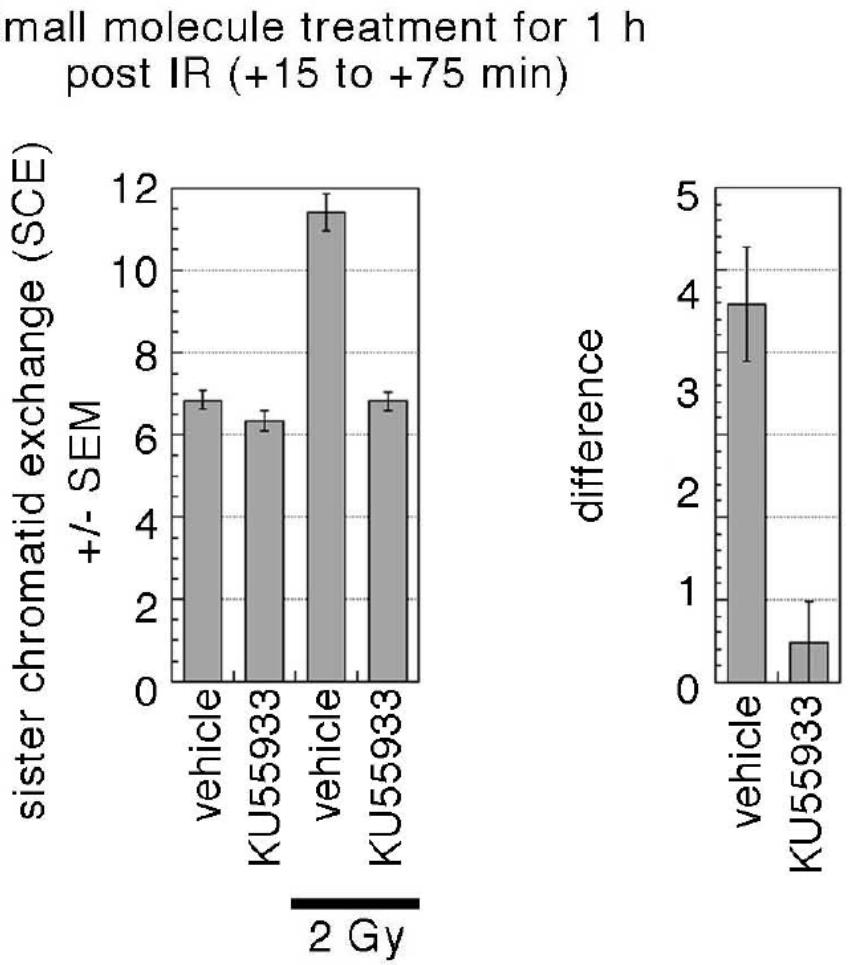
Sister Chromatid Exchange (SCE)



Sister chromatid exchanges following exposure to 2 Gy and ATM kinase inhibition for 1h post-IR

Transient inhibition of ATM kinase activity is sufficient to abrogate IR-induced sister chromatid exchange





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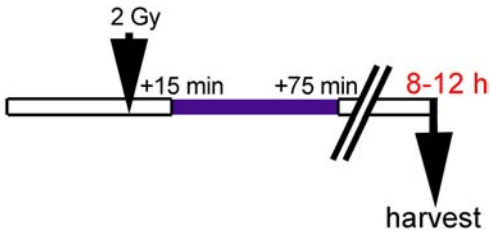
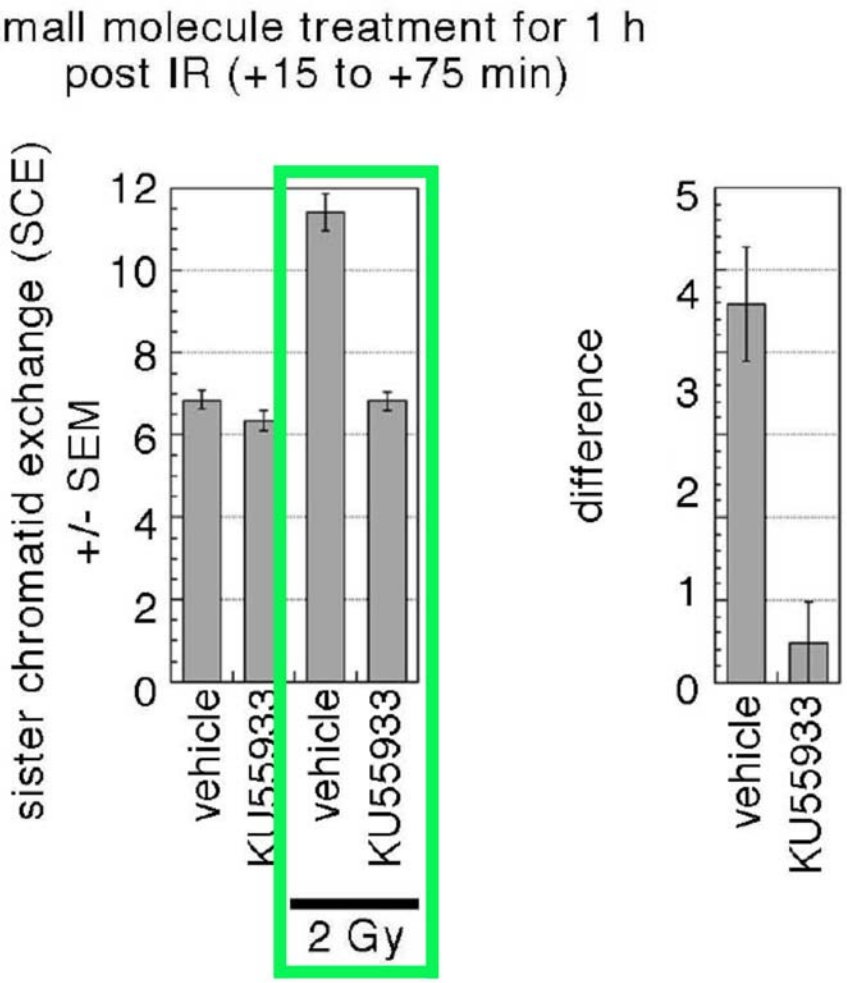
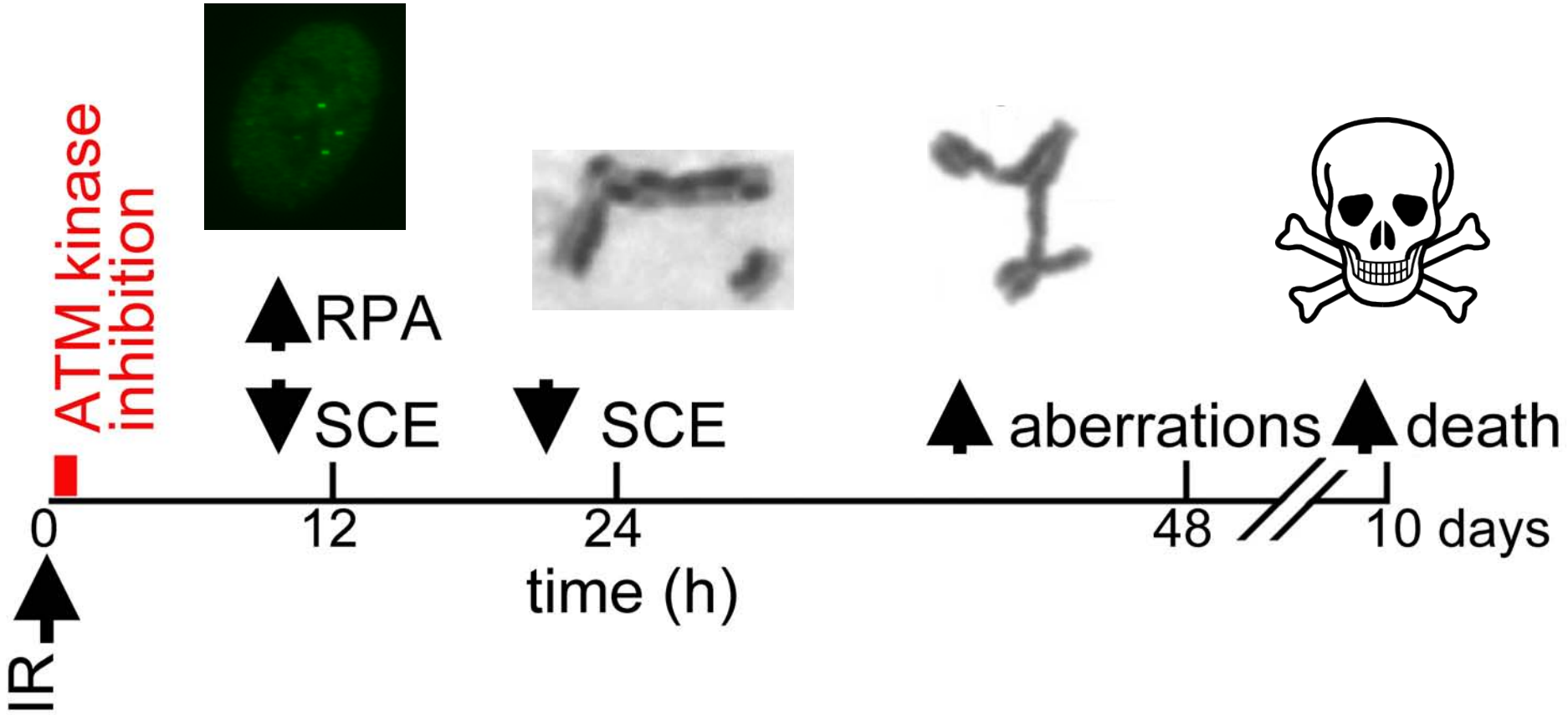




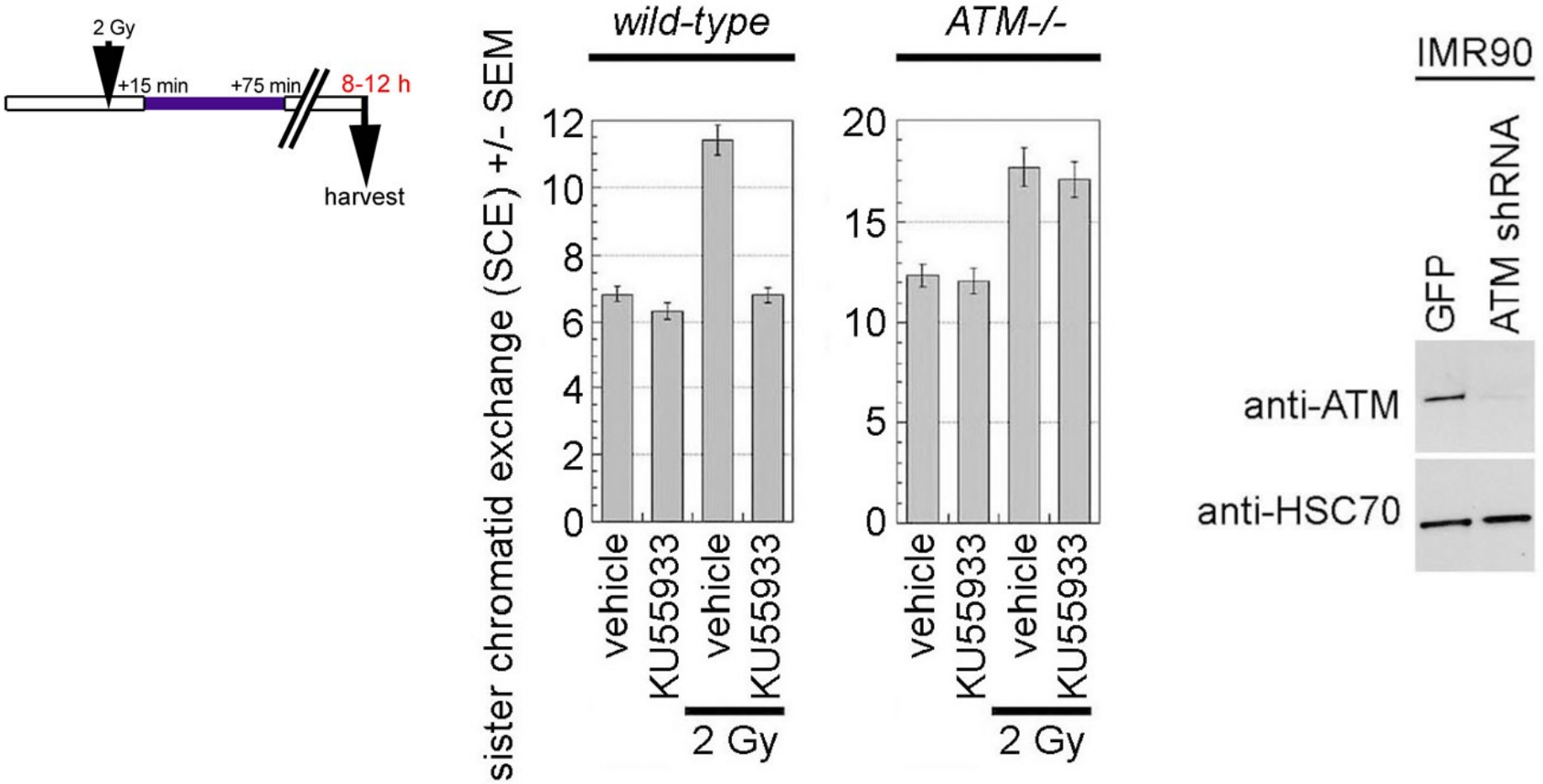
White JS, Choi S, Bakkenist CJ (2008) Cell Cycle 7, 1277-1284

Irreversible damage accumulates rapidly when ATM kinase is inhibited



Choi S, White JS, Bakkenist CJ (2010) Cell Cycle 9, 4052-4057

Chemically-inhibited ATM disrupts SCE in a manner that does not occur in the absence of ATM protein



White JS, Choi S, Bakkenist CJ (2010) Science Signaling 3, ra44

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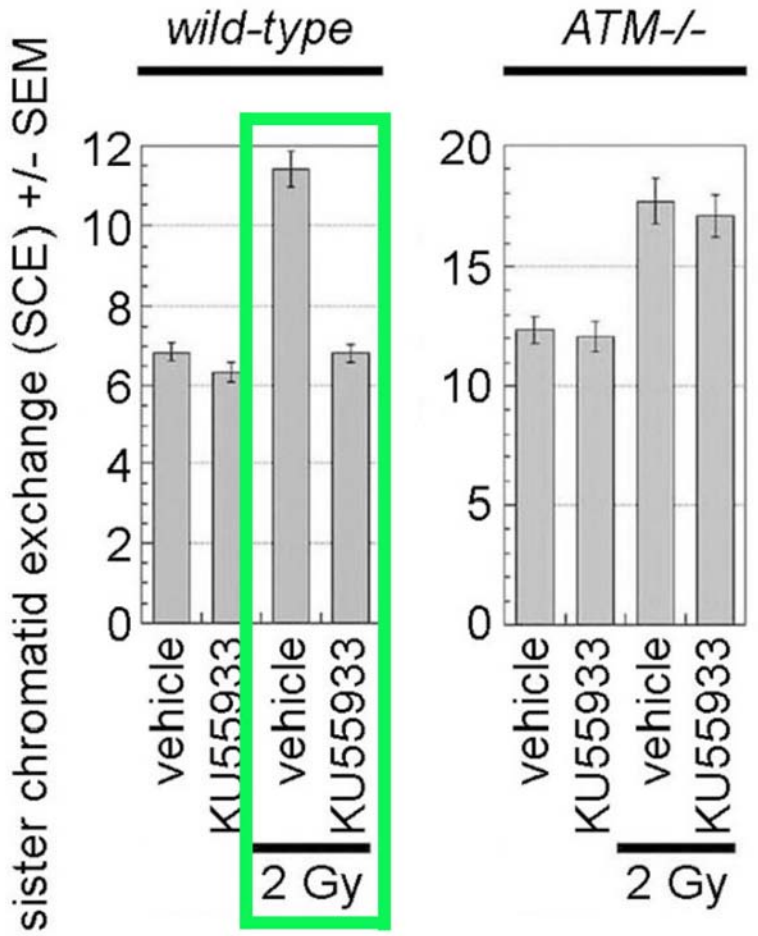

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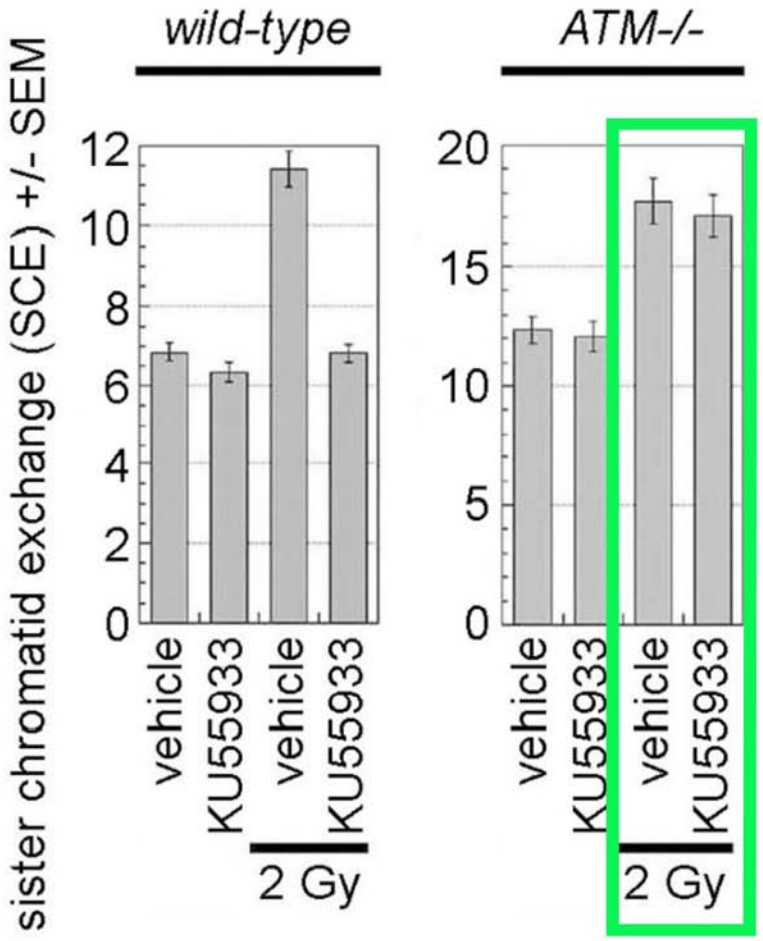

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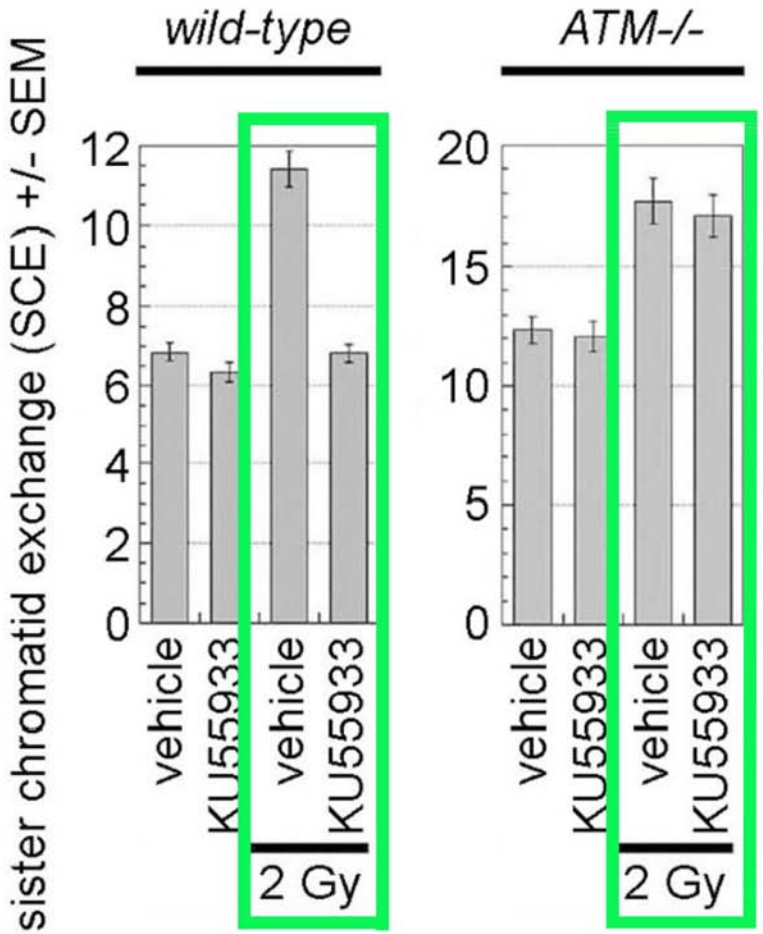

This is not an off-target effect

It is ATM-dependent

White JS, Choi S, Bakkenist CJ (2010) Science Signaling 3, ra44

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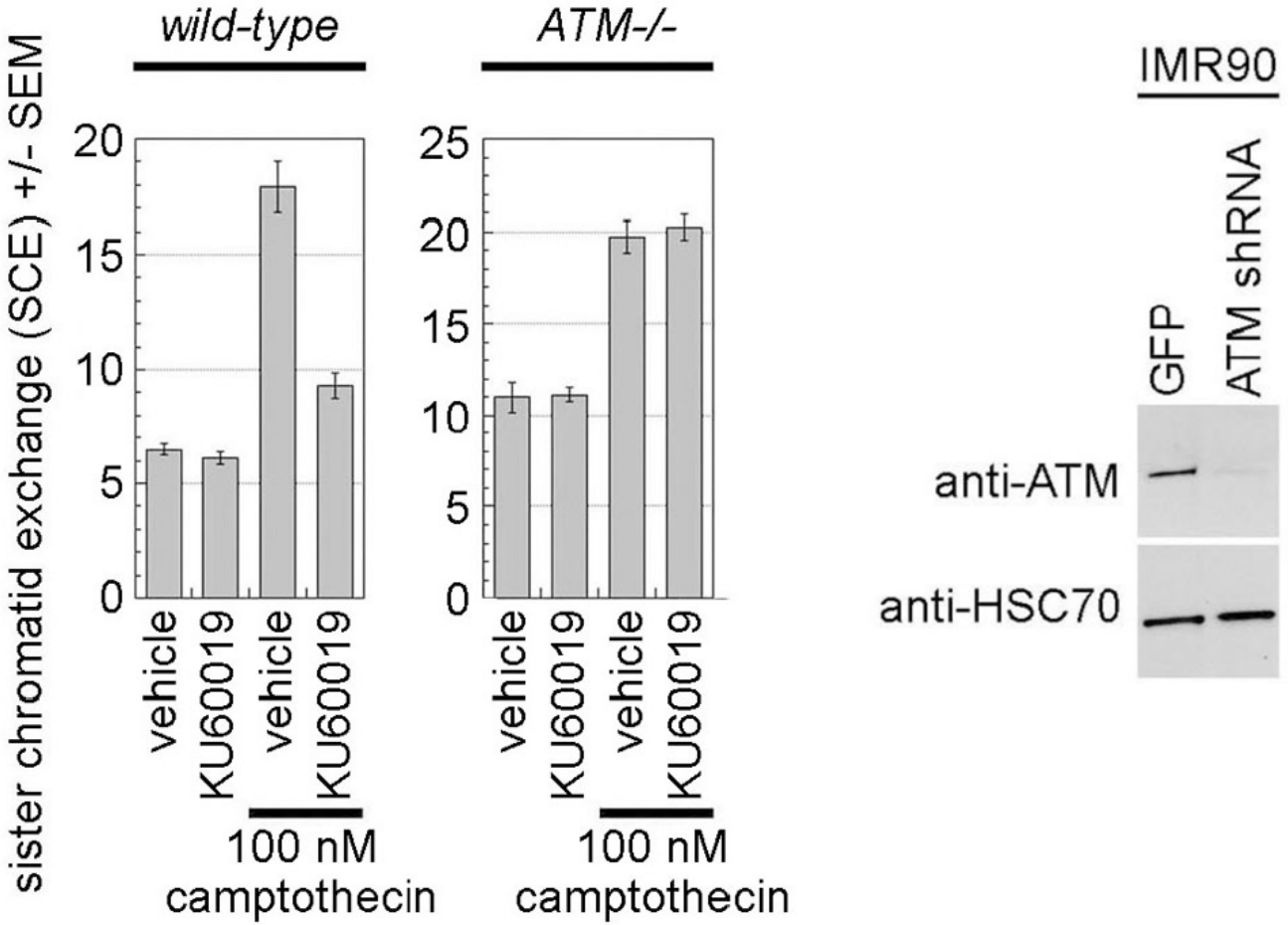

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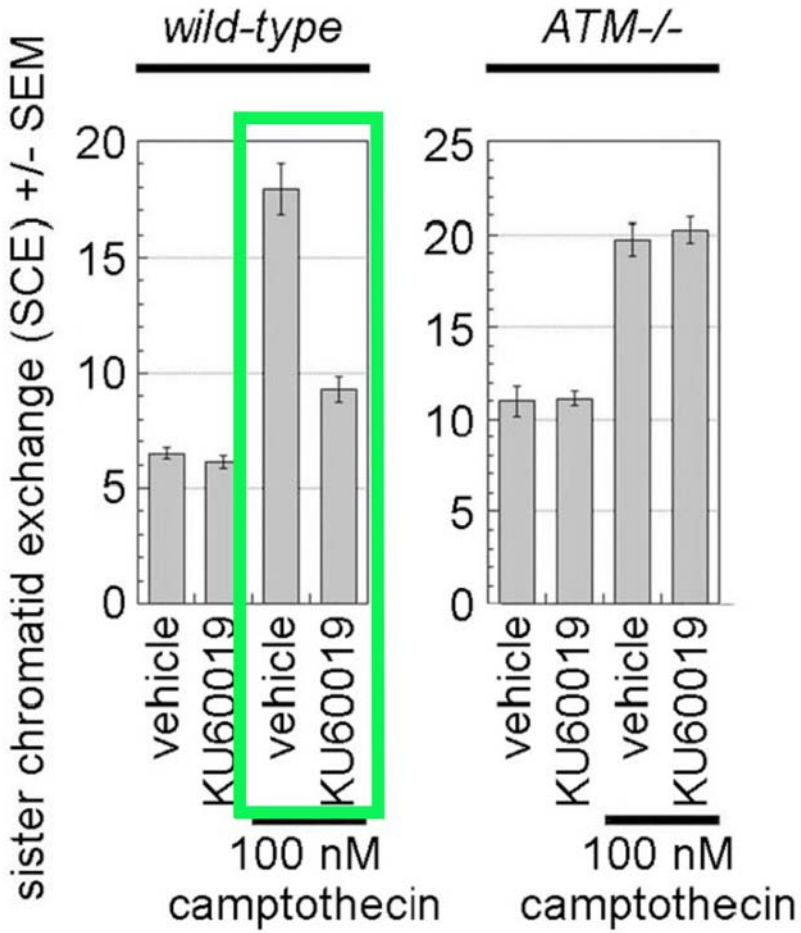

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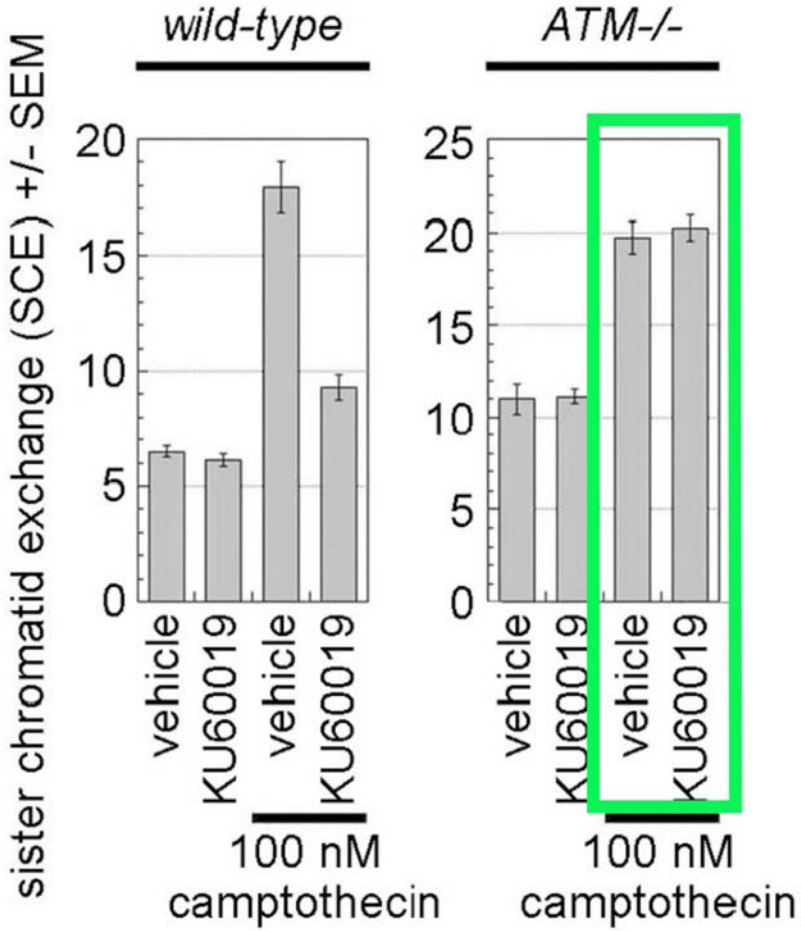

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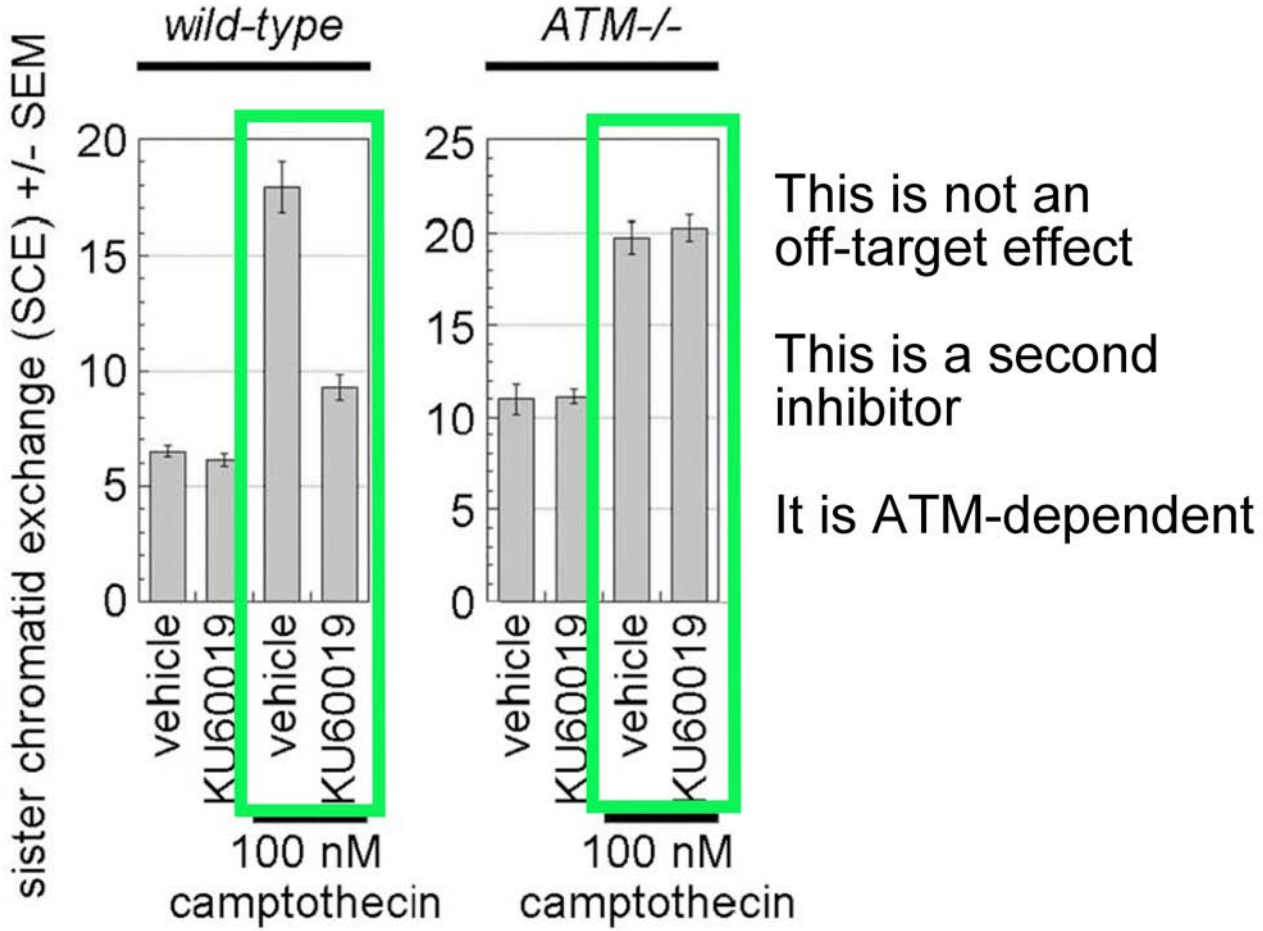


White JS, Choi S, Bakkenist CJ (2010) Science Signaling 3, ra44

ATM kinase-inactive does not phenocopy ATM protein disruption

no ATM	chemically-inhibited ATM
SCE ✓	SCE ✗

(Bakkenist, Maria Jasin labs)

White JS, Choi S, Bakkenist CJ (2010) Science Signaling 3, ra44

Choi S, White JS, Bakkenist CJ (2010) Cell Cycle 9, 4052-4057

3,000 Americans

300,000,000 Americans

ATM kinase-inactive does not phenocopy ATM protein disruption

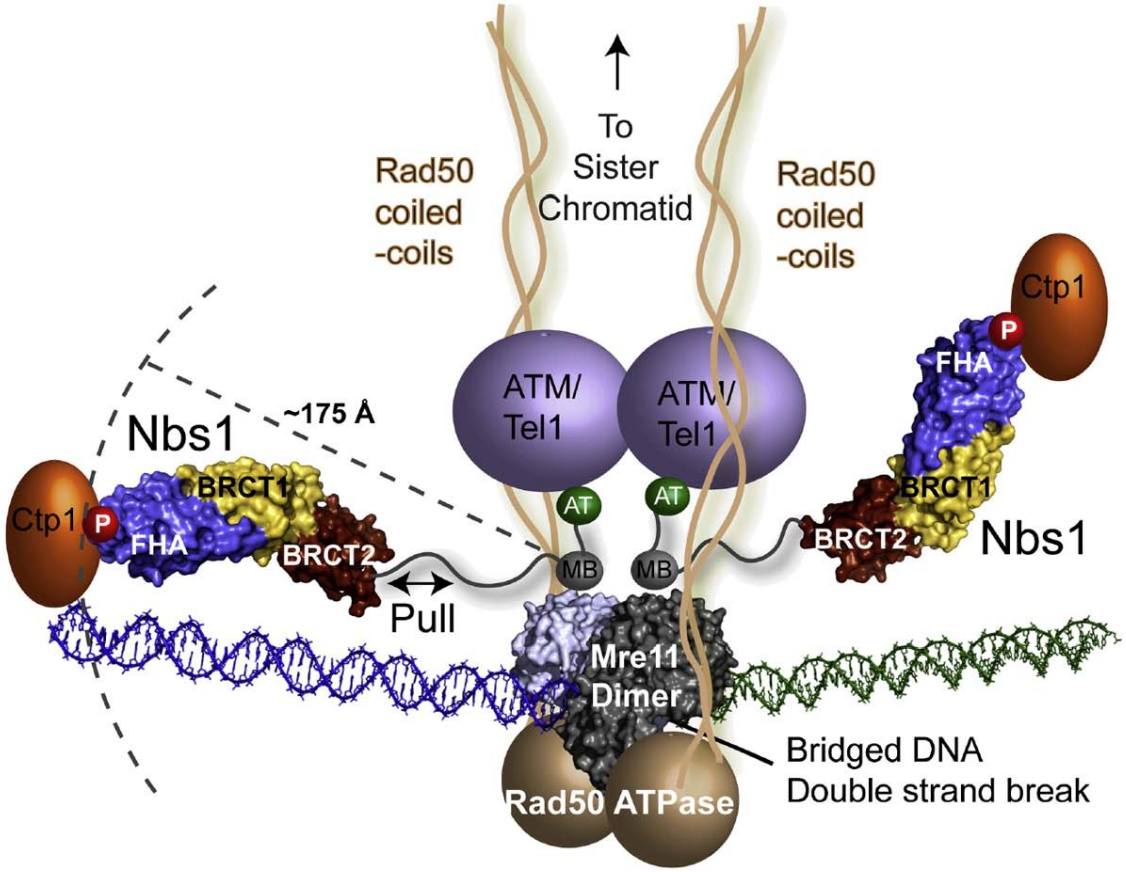
no ATM

viable

ATM, but kinase-inactive

embryonic lethal
(Nussenzweig, Shan Zha labs)

Chemically-inhibited ATM disrupts SCE in a manner that does not occur in the absence of ATM protein

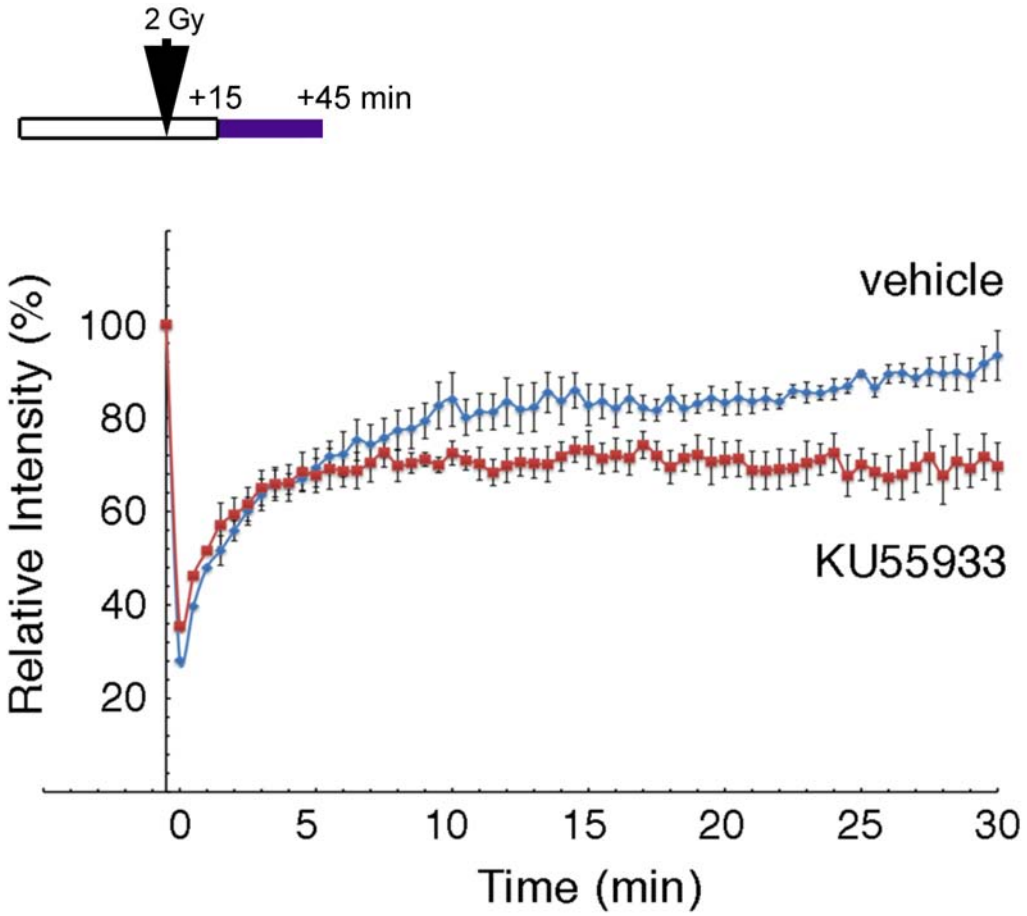


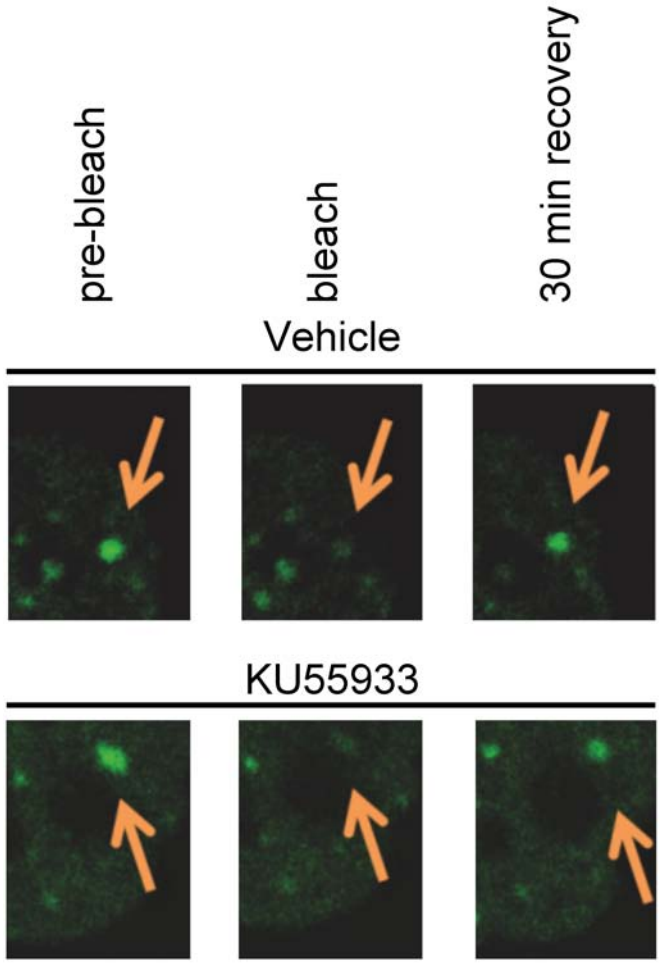
- NBS1 appears to be a hub that promotes the integration of DNA repair activities by interface exchange and handoff interactions with multiple partners
- We hypothesize that ATM kinase is positioned to promote, or when inhibited, block these exchanges.

Williams RS et. al. (2009) Cell 139, 87-99

ATM kinase inhibition at +15 post-IR disrupts 53BP1 foci

FRAP with Simon Watkins





Choi et al., in preparation

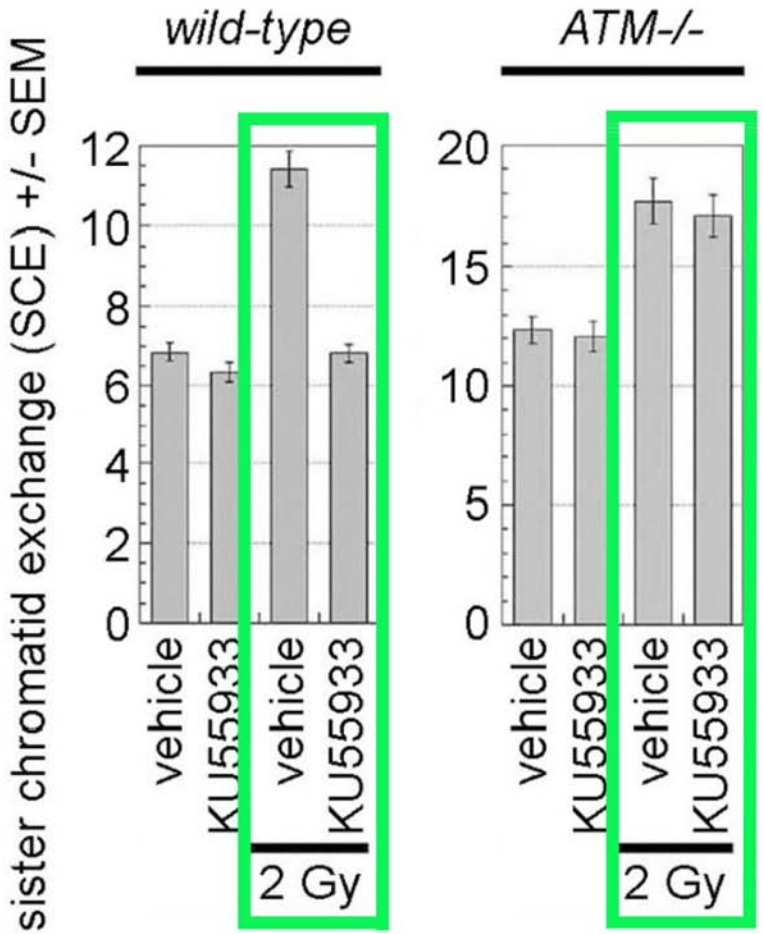
Summary Section 2

Irreversible damage accumulates rapidly when ATM kinase activity is inhibited in irradiated cells

Chemically-inhibited ATM kinase blocks DNA repair in a manner that does not occur in the absence of ATM protein

300,000,000 Americans have ATM
3,000 do not

Chemically-inhibited ATM disrupts SCE in a manner that does not occur in the absence of ATM protein



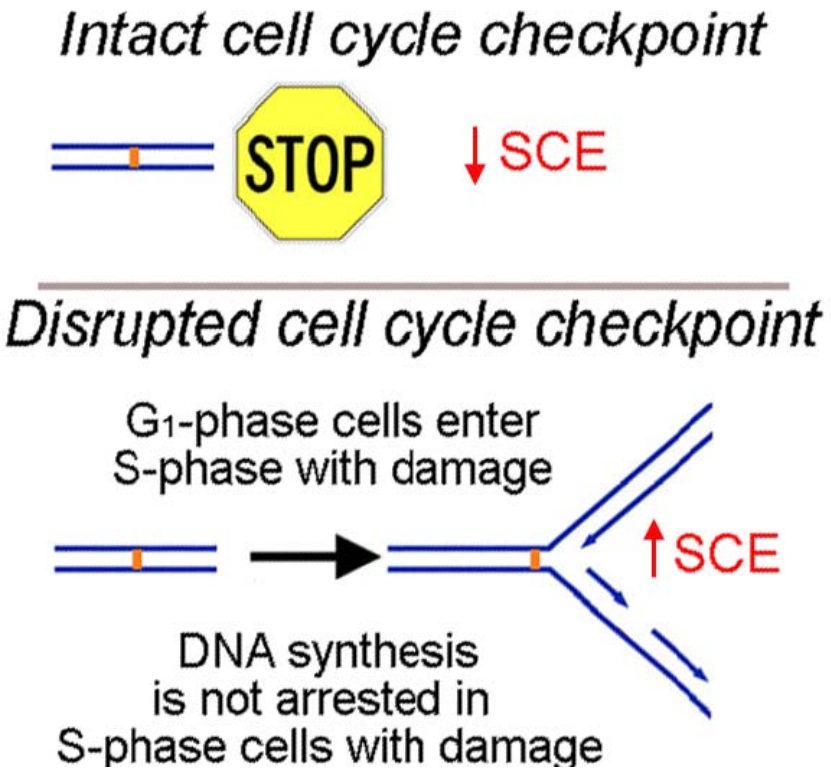
This is not an off-target effect

It is ATM-dependent

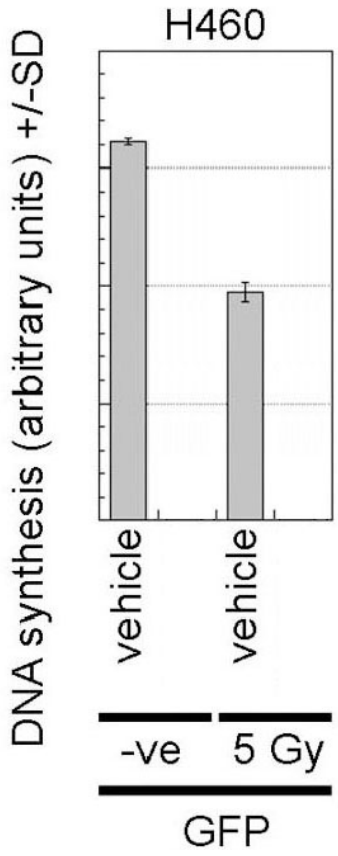
White JS, Choi S, Bakkenist CJ (2010) Science Signaling 3, ra44

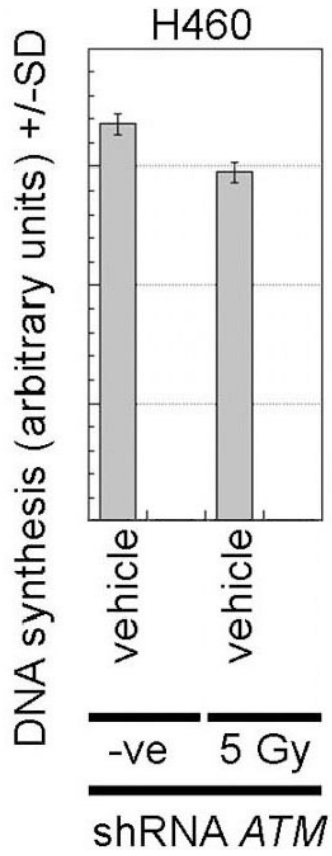
Radio-resistant DNA synthesis (RDS)

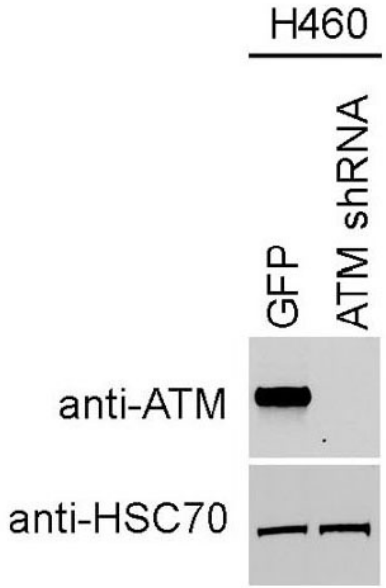
- DNA synthesis is not inhibited following irradiation in A-T cells due to a failure to inhibit late-origin firing



Radio-resistant DNA synthesis







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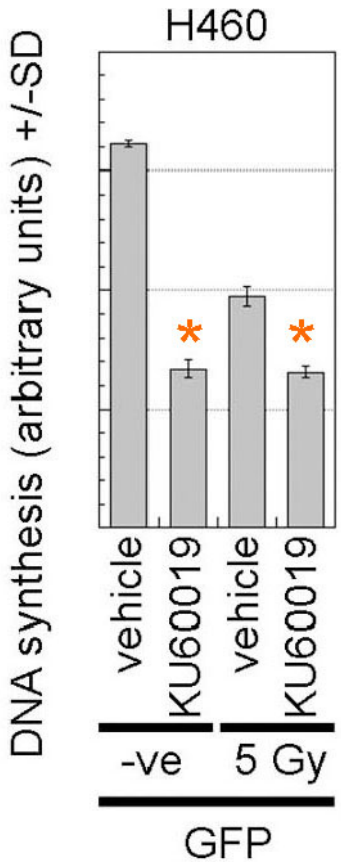
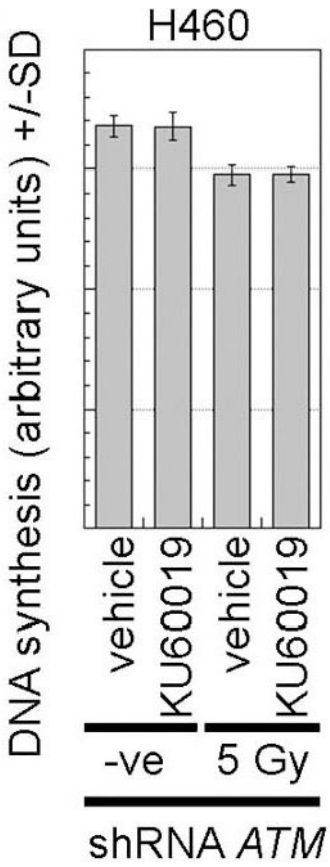
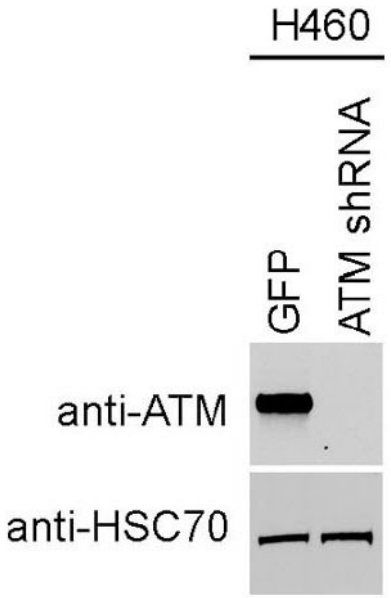
DNA synthesis is disrupted by ATM kinase inhibition







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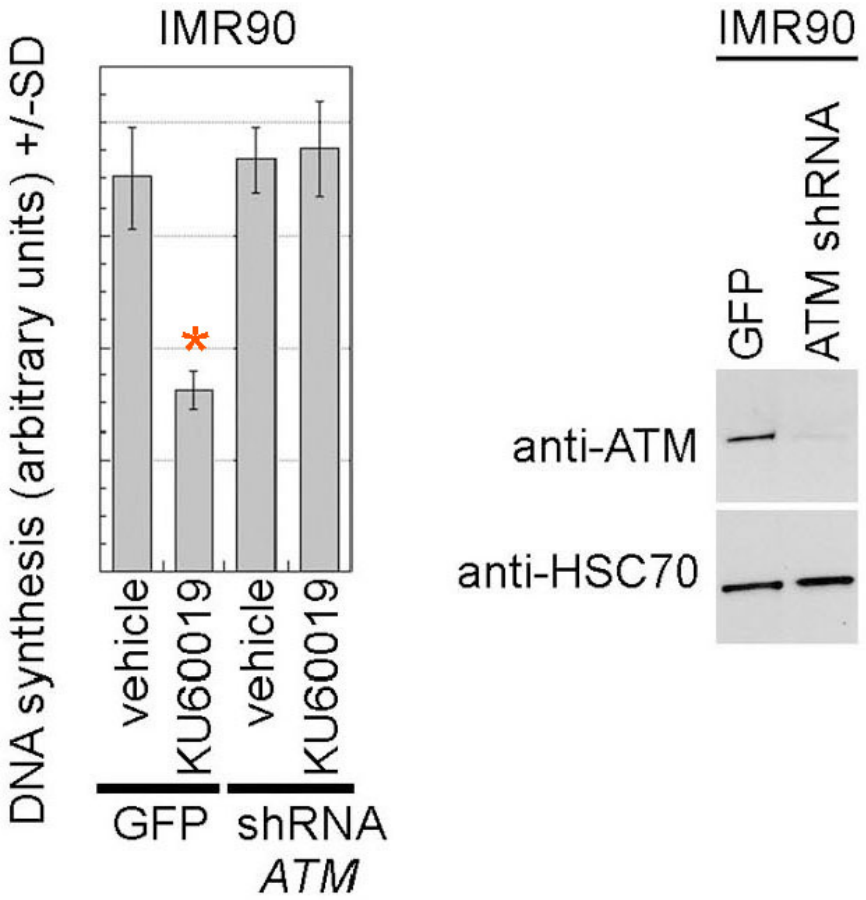
DNA synthesis is disrupted by ATM kinase inhibition



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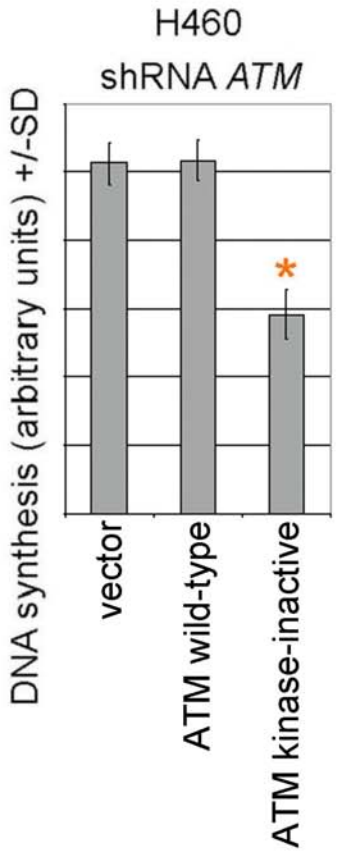
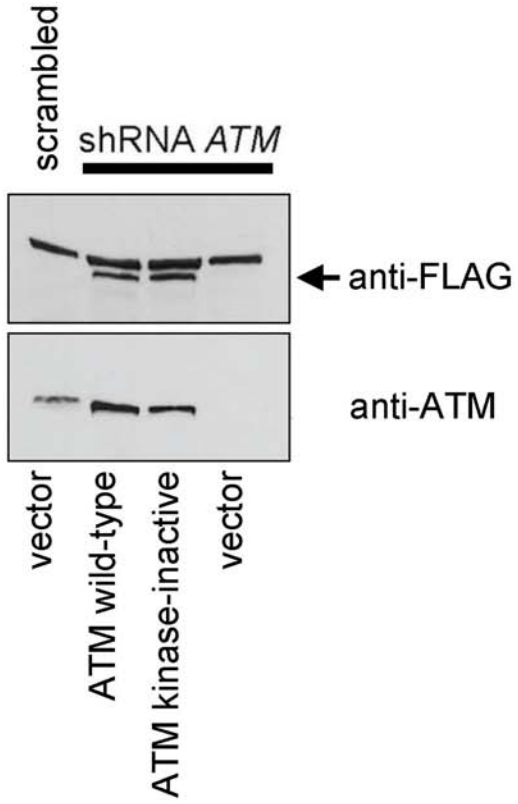
DNA synthesis is disrupted by ATM kinase inactive expression in cells expressing shRNA that knockdowns endogenous ATM





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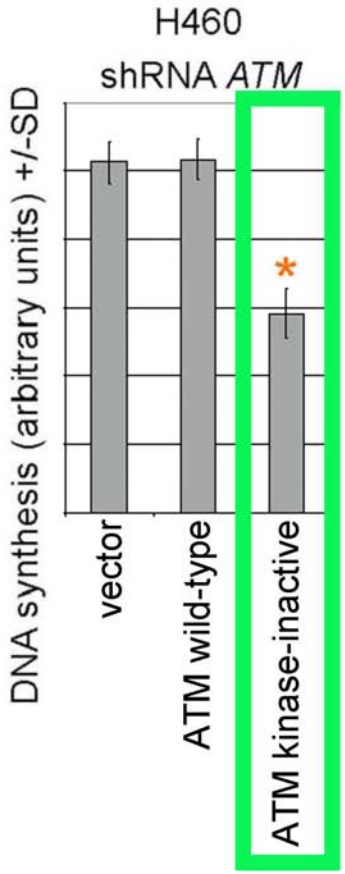

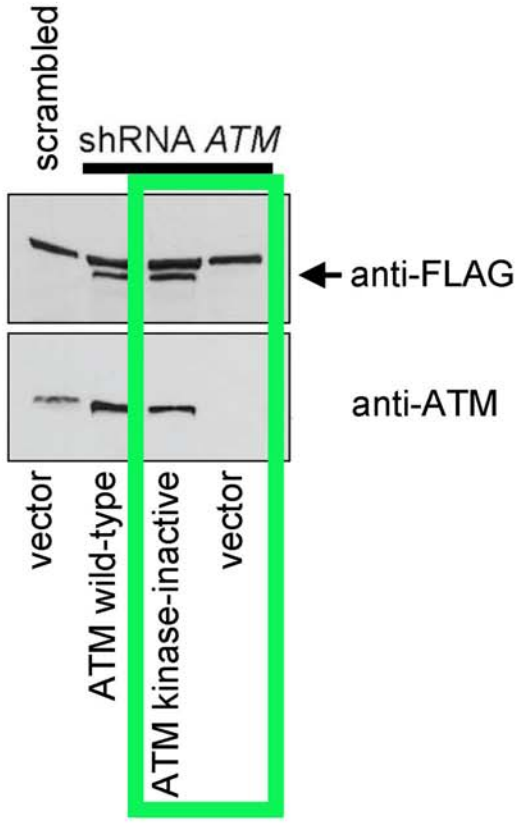

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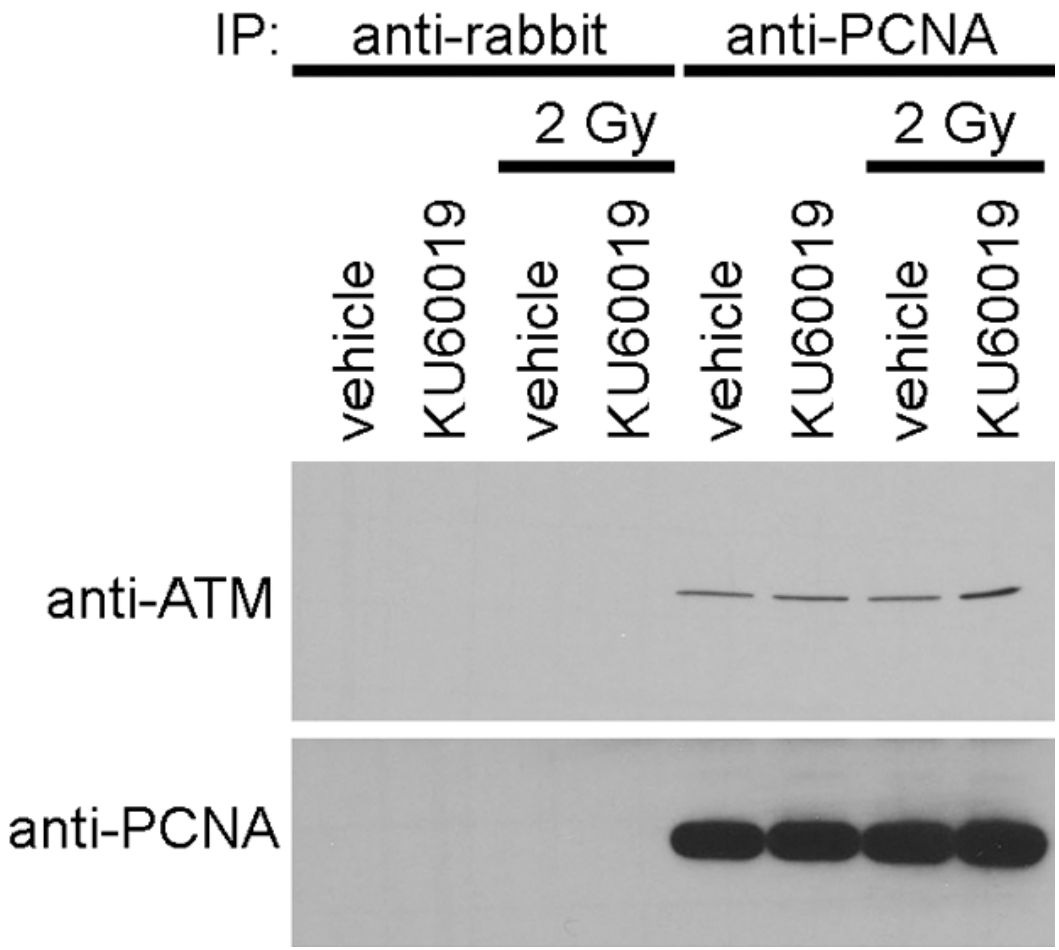
PCNA interacts with ATM in cells - Serah Choi



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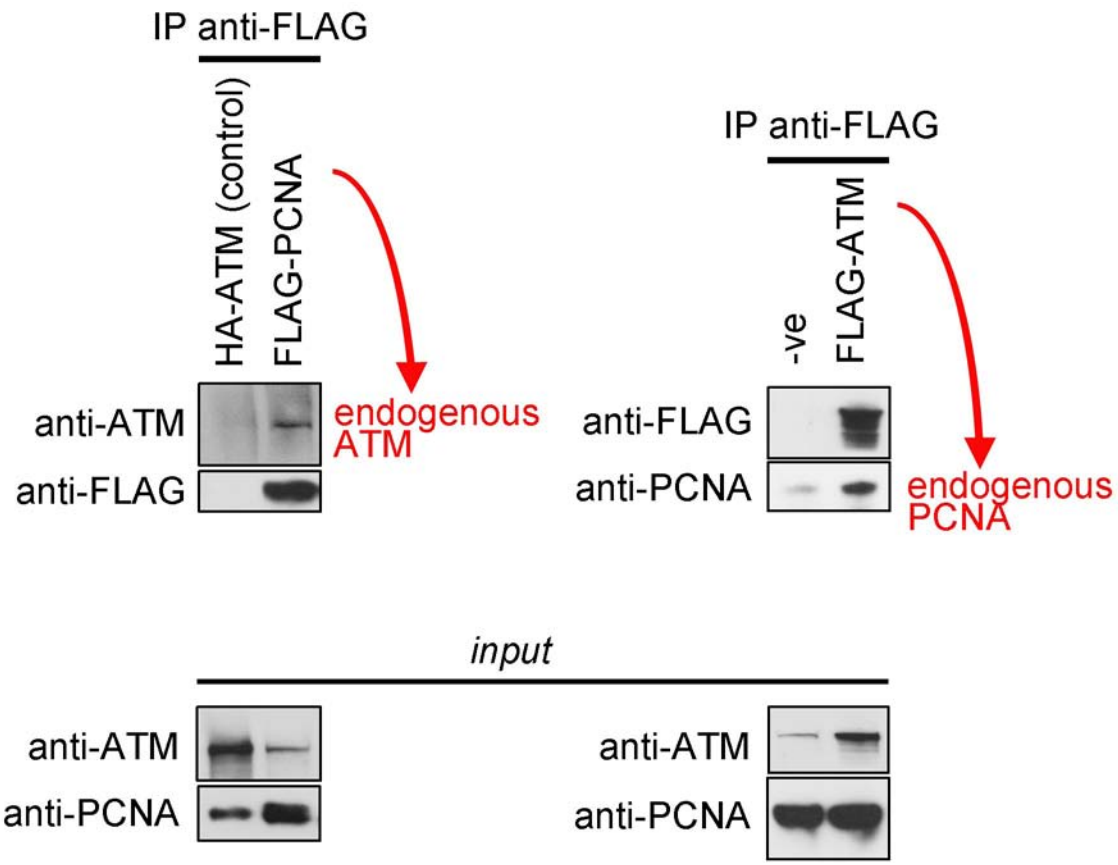
PCNA interacts with ATM in cells



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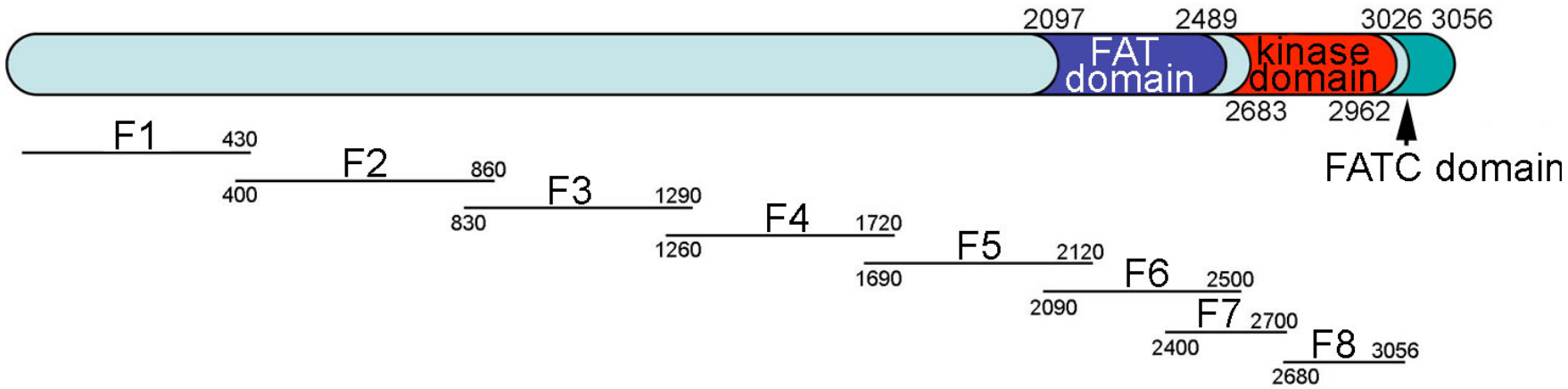
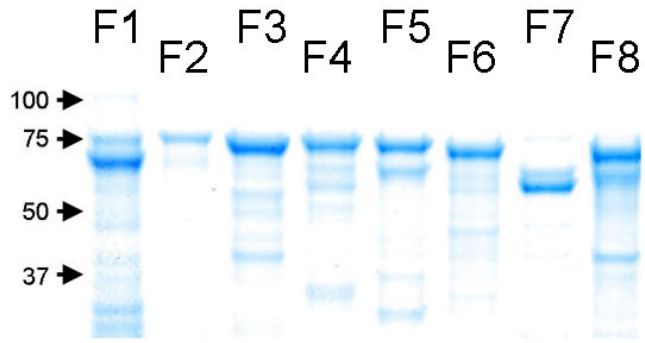
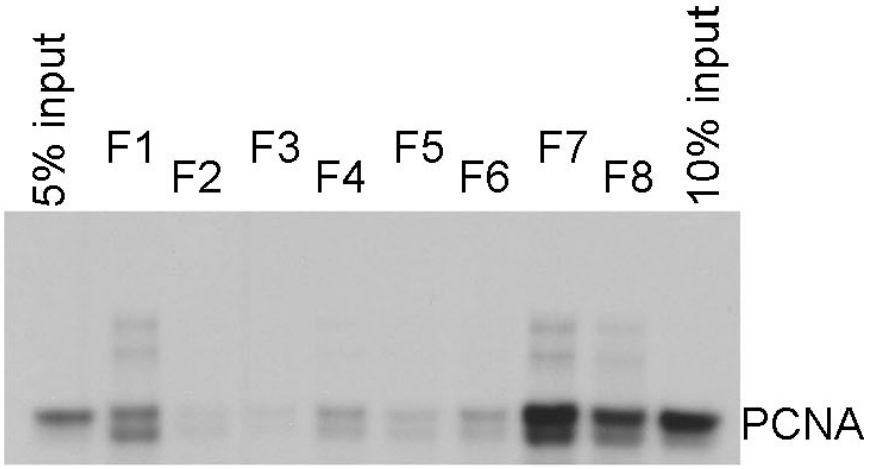
PCNA interacts with ATM *in vitro*







Thank you Dr. Titia de Lange

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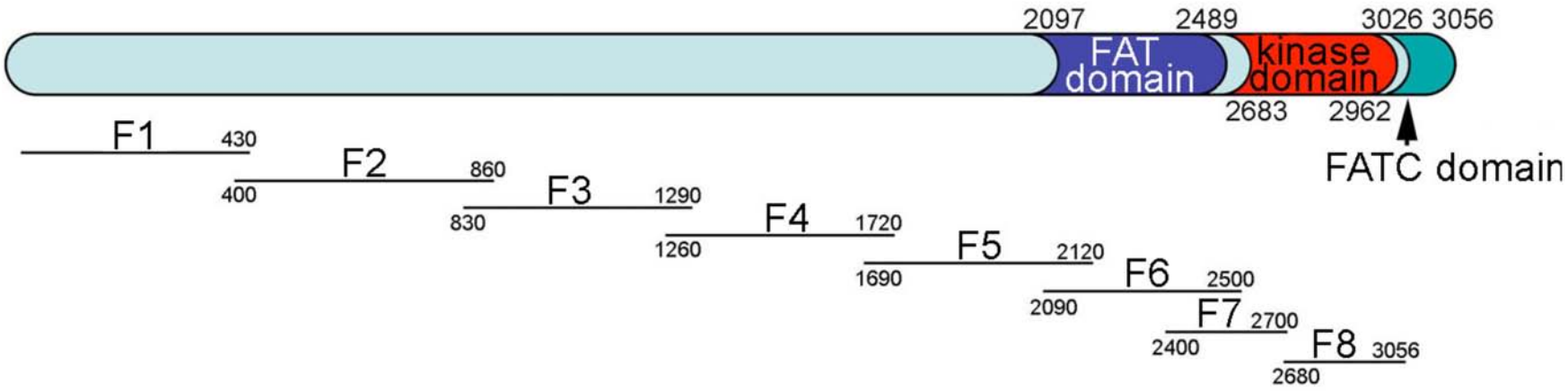
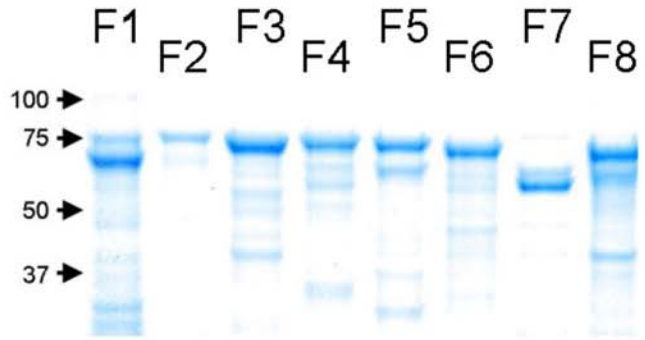
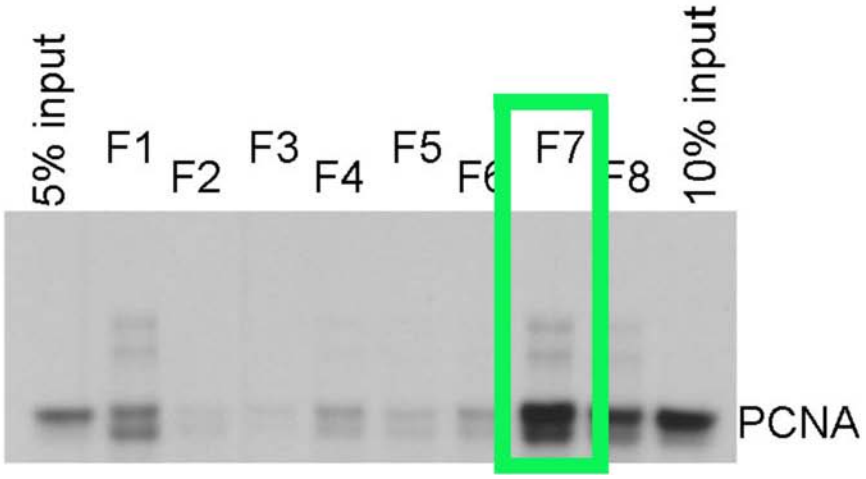
PCNA interacts with ATM *in vitro*







Thank you Dr. Titia de Lange

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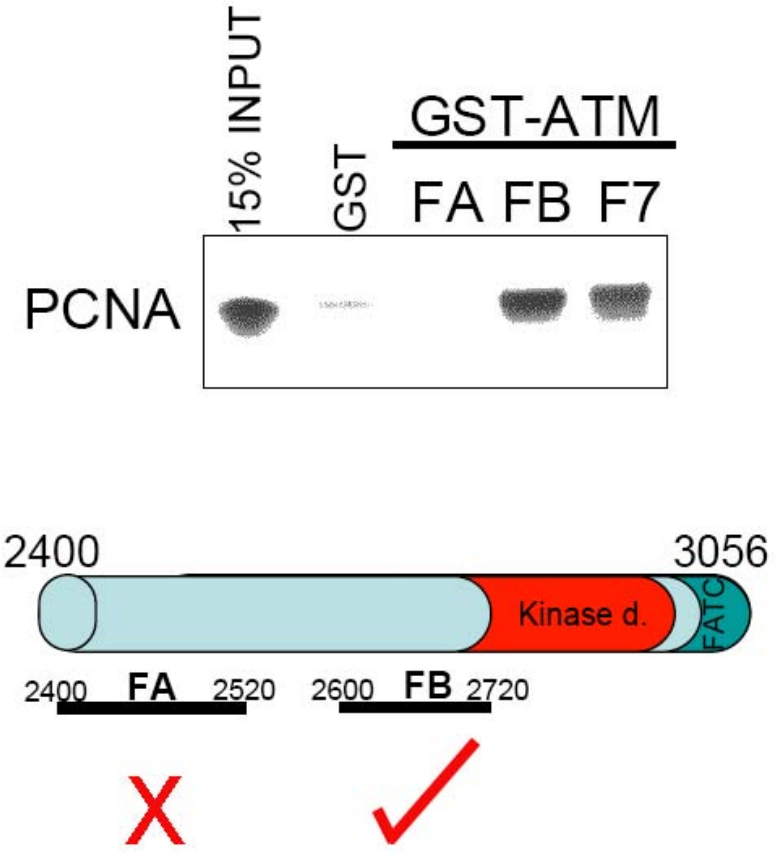
Fine-Mapping the Interaction of ATM with PCNA



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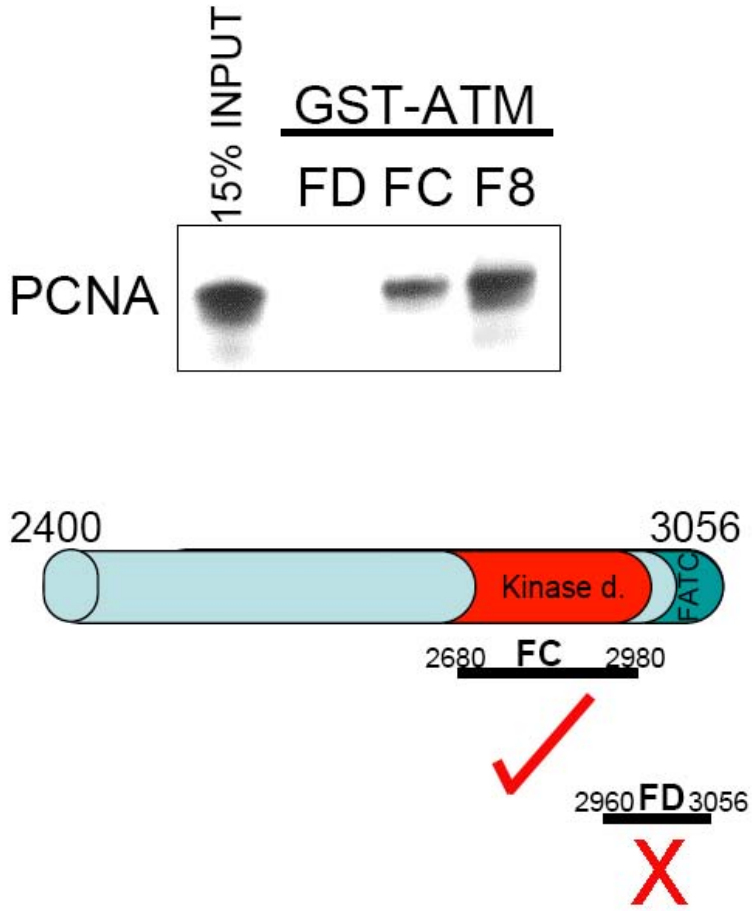
Fine-Mapping the Interaction of ATM with PCNA



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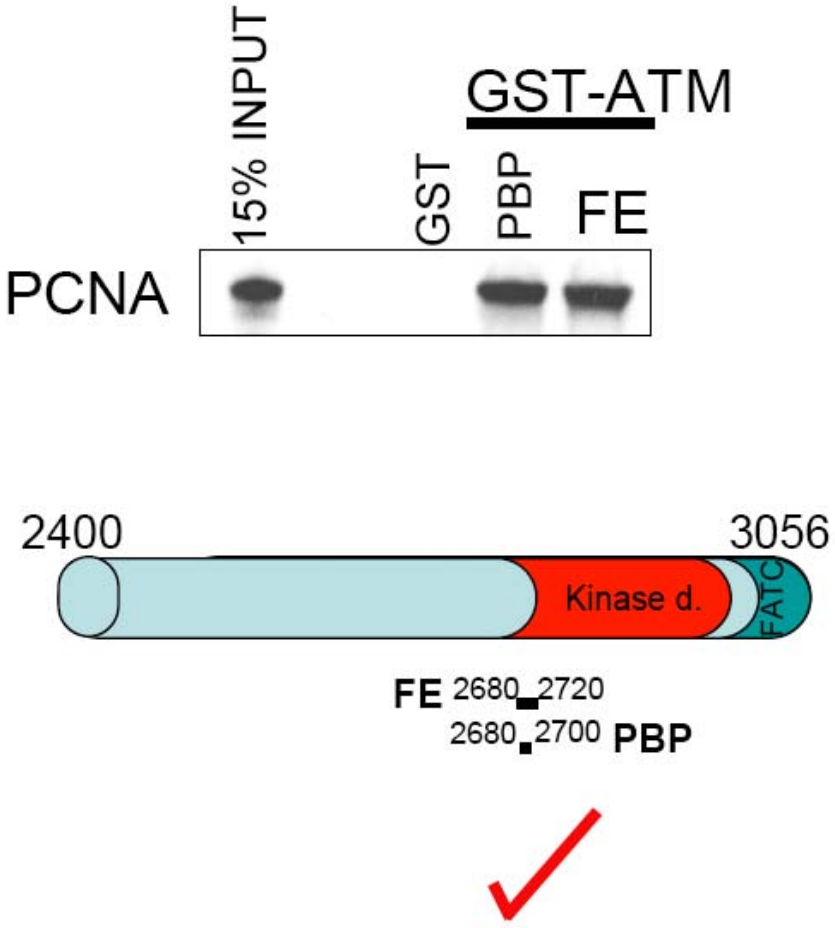
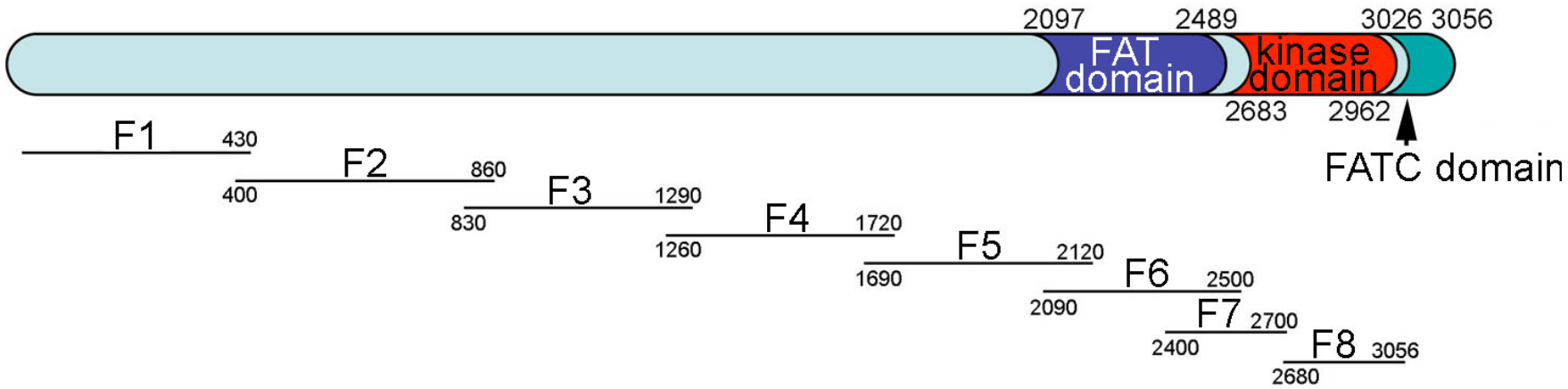
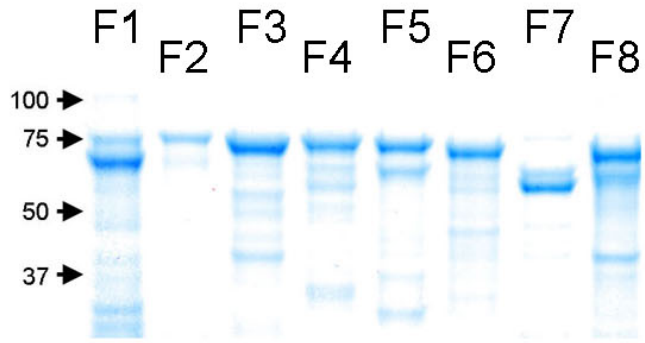
Fine-Mapping the Interaction of ATM with PCNA

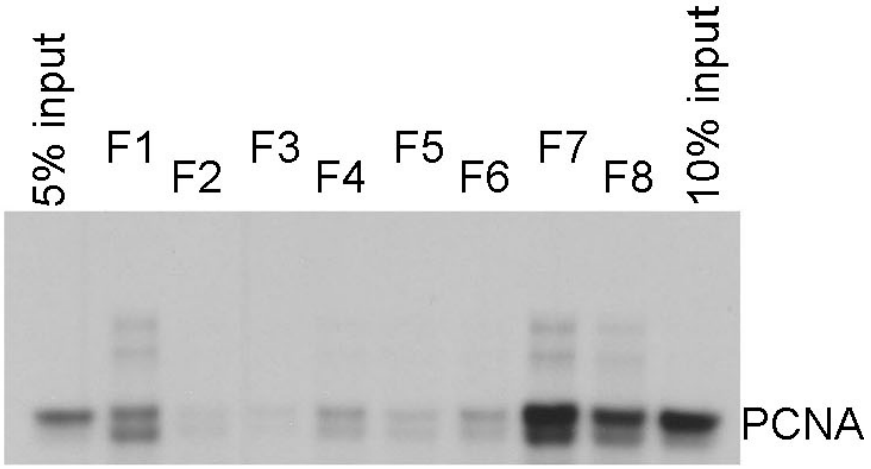


Gamper, Choi, et al. in review

PCNA interacts with ATM *in vitro*







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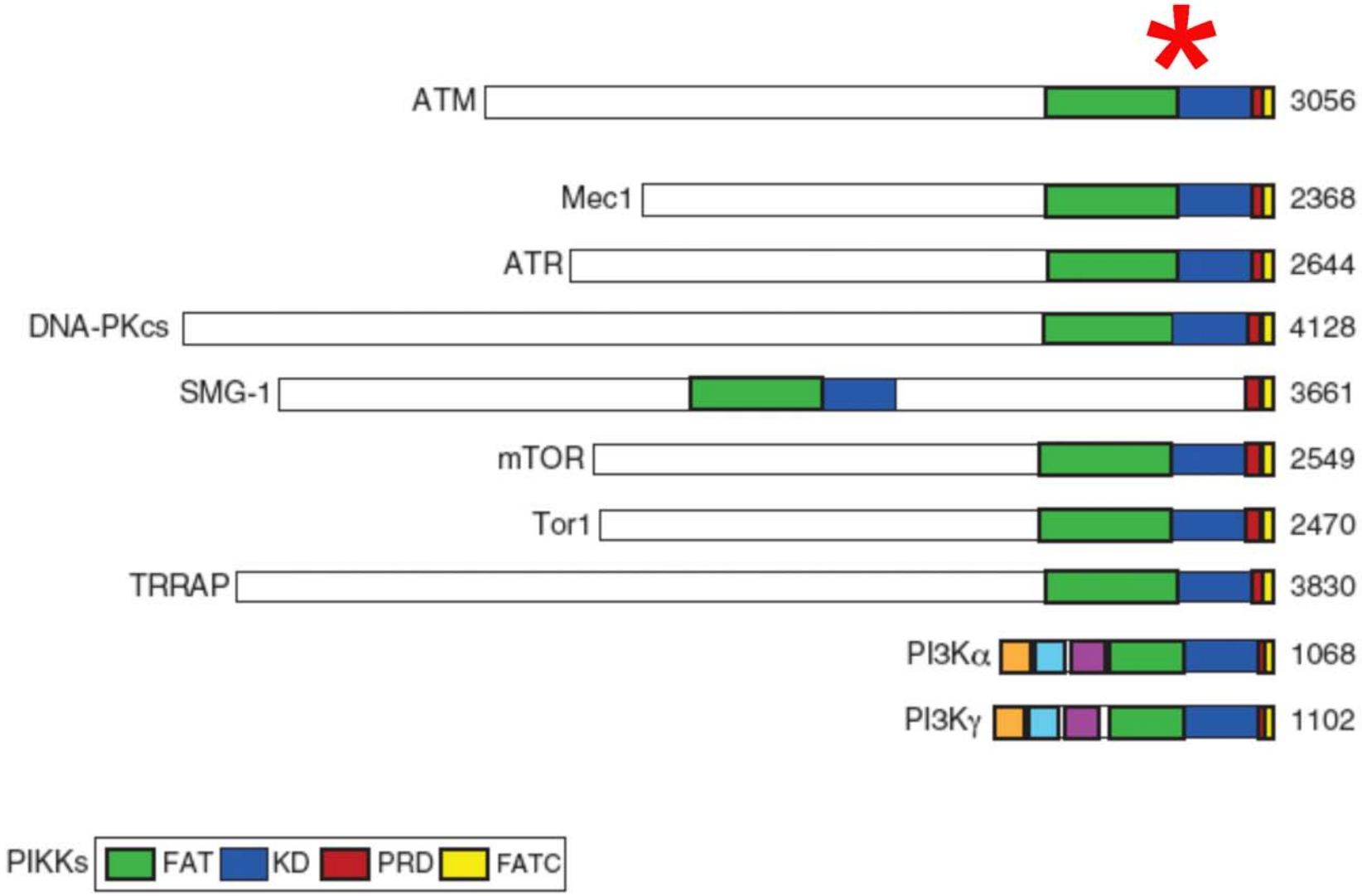
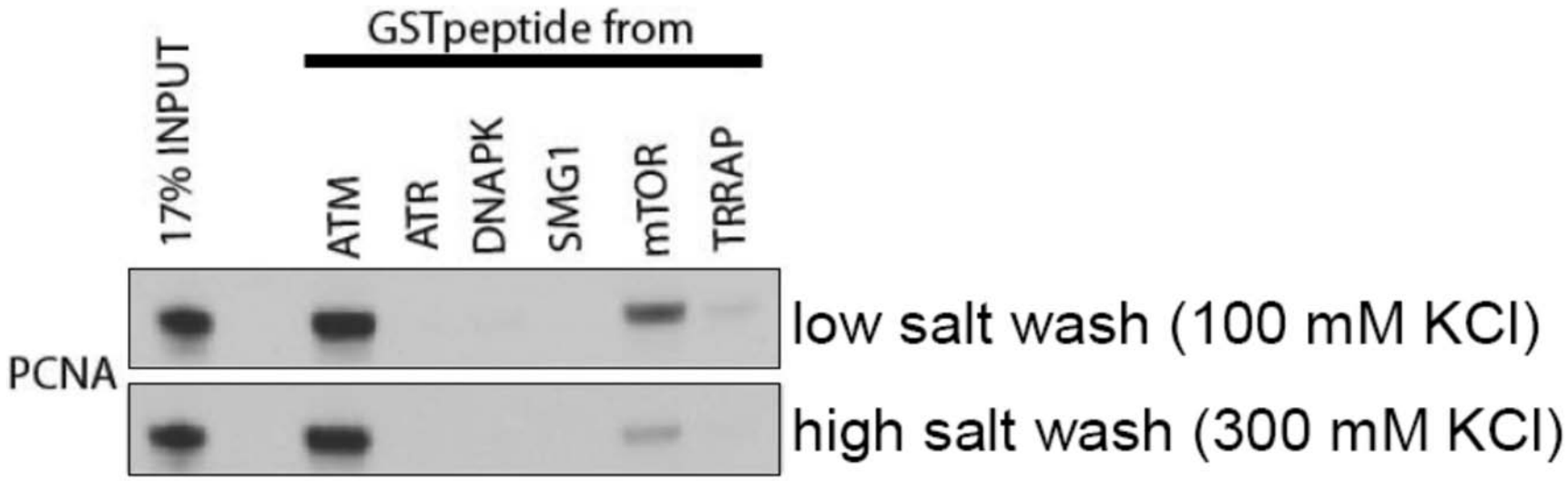
The PIKK/PI3K family



Lempiäinen H and Halazonetis TD (2009) EMBO J 28, 3067-3073

The interaction of PCNA with ATM is specific



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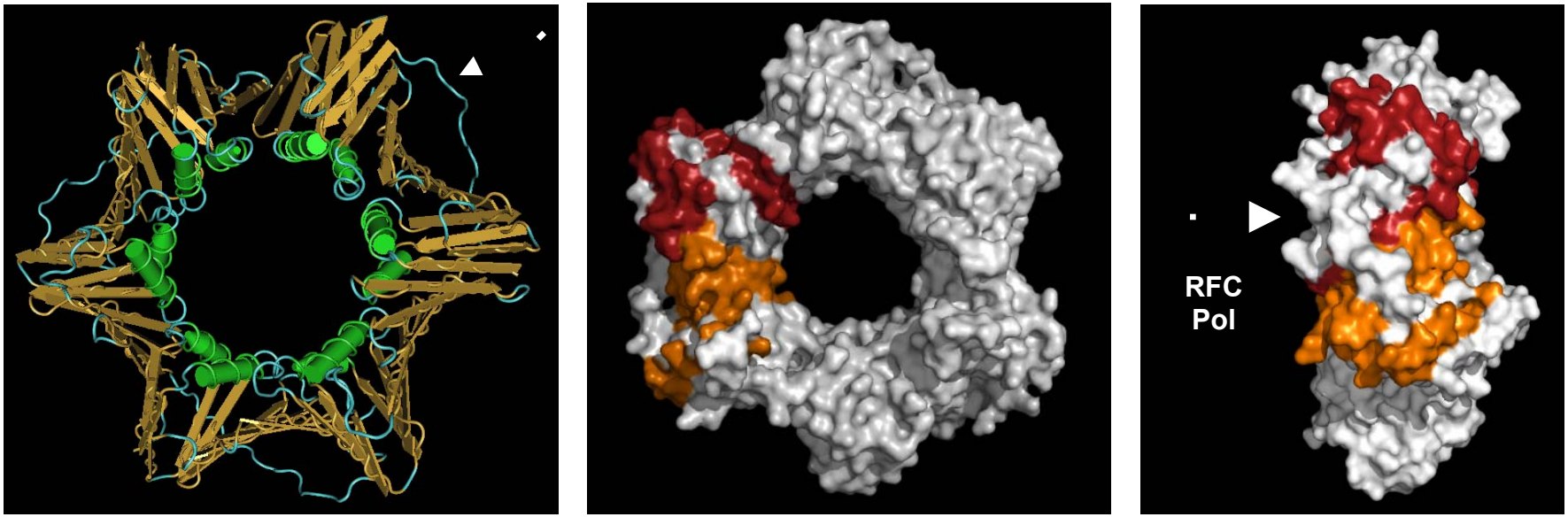
Fine-Mapping the interaction of PCNA with ATM



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Summary Section 3

ATM interacts with PCNA *in vivo* and *in vitro*

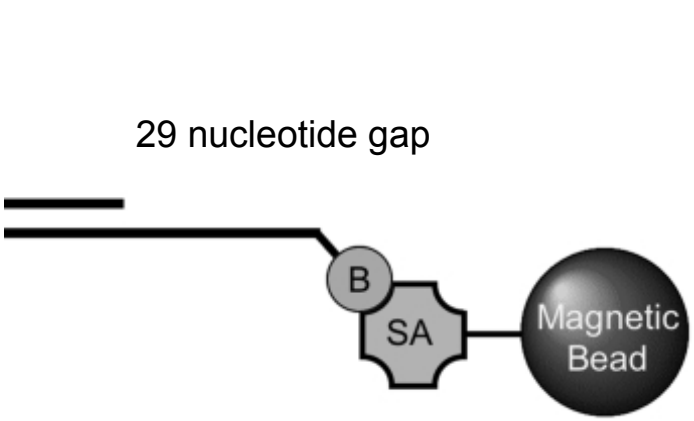
A 20 amino acid region of ATM is sufficient to bind PCNA

PCNA binding to ATM is not mediated by the PCNA interdomain loop

Is there any functional significance of the binding?

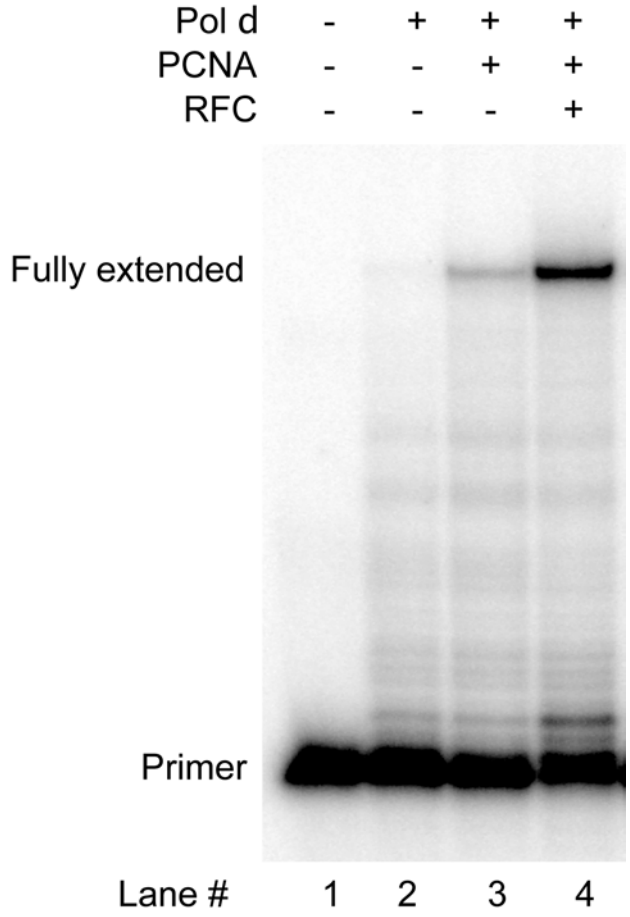
ATM, PCNA and DNA synthesis

with Alan Tomkinson



DNA polymerase delta
PCNA
+/- RFC
γ^{32}P ATP primer

primer extension



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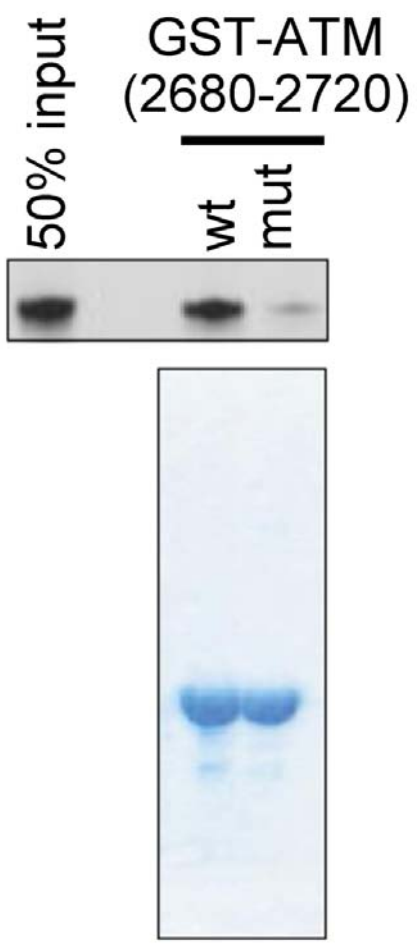
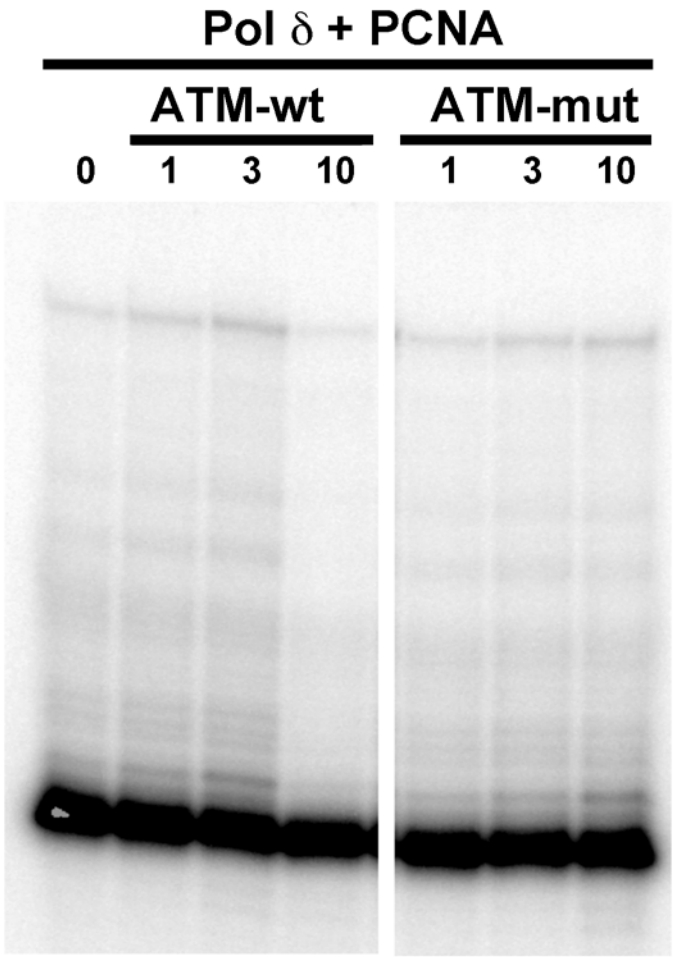
ATM, PCNA (but no RFC) and DNA synthesis

with Alan Tomkinson

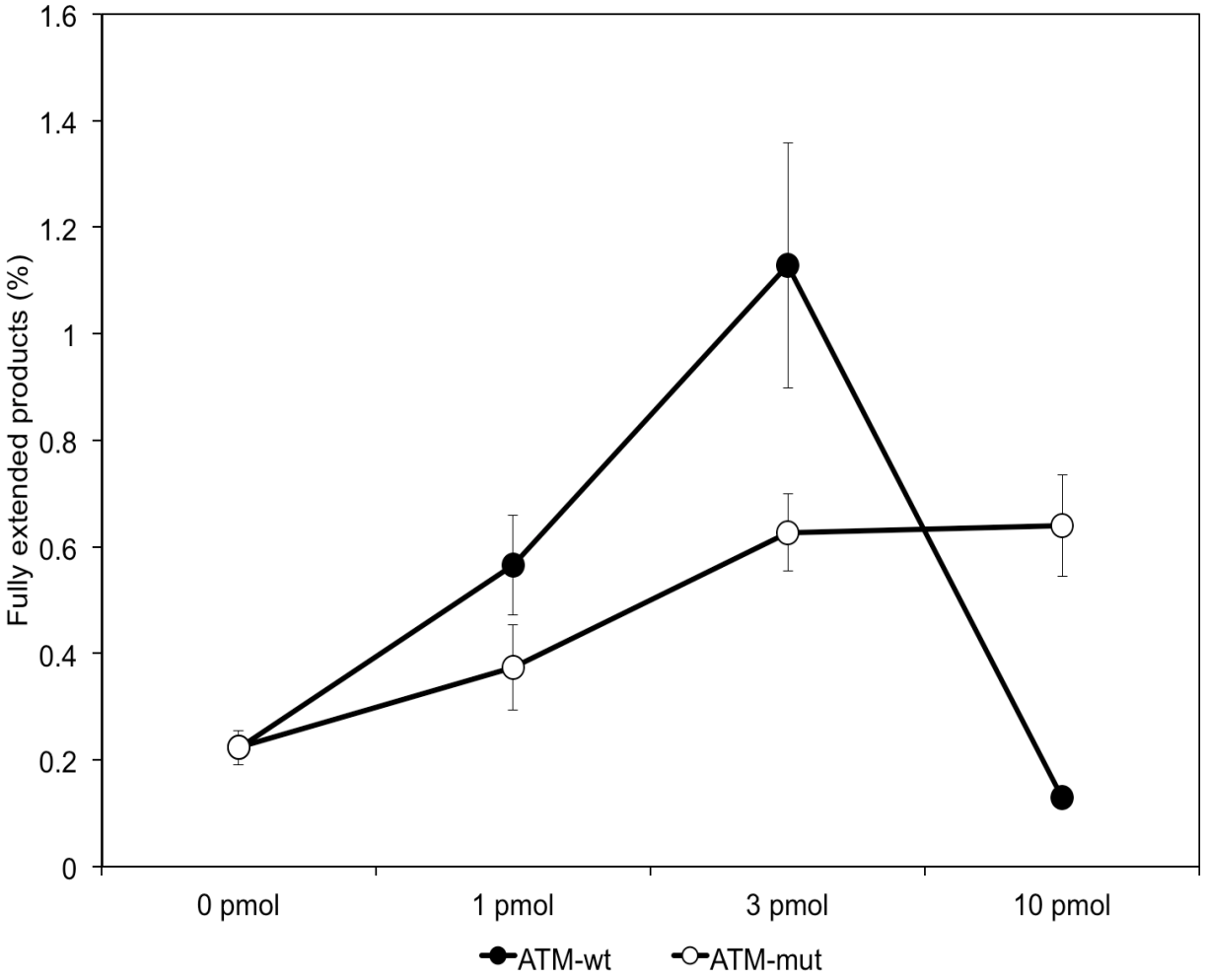




Gamper, Choi, et al. in review

ATM, PCNA (but no RFC) and DNA synthesis

with Alan Tomkinson



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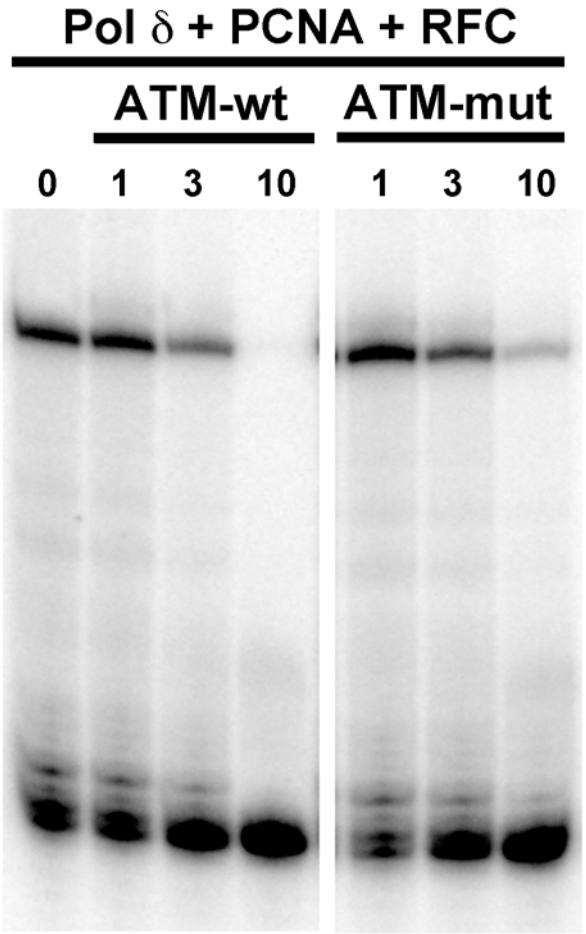
ATM, PCNA, RFC and DNA synthesis

with Alan Tomkinson



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ATM, PCNA, RFC and DNA synthesis

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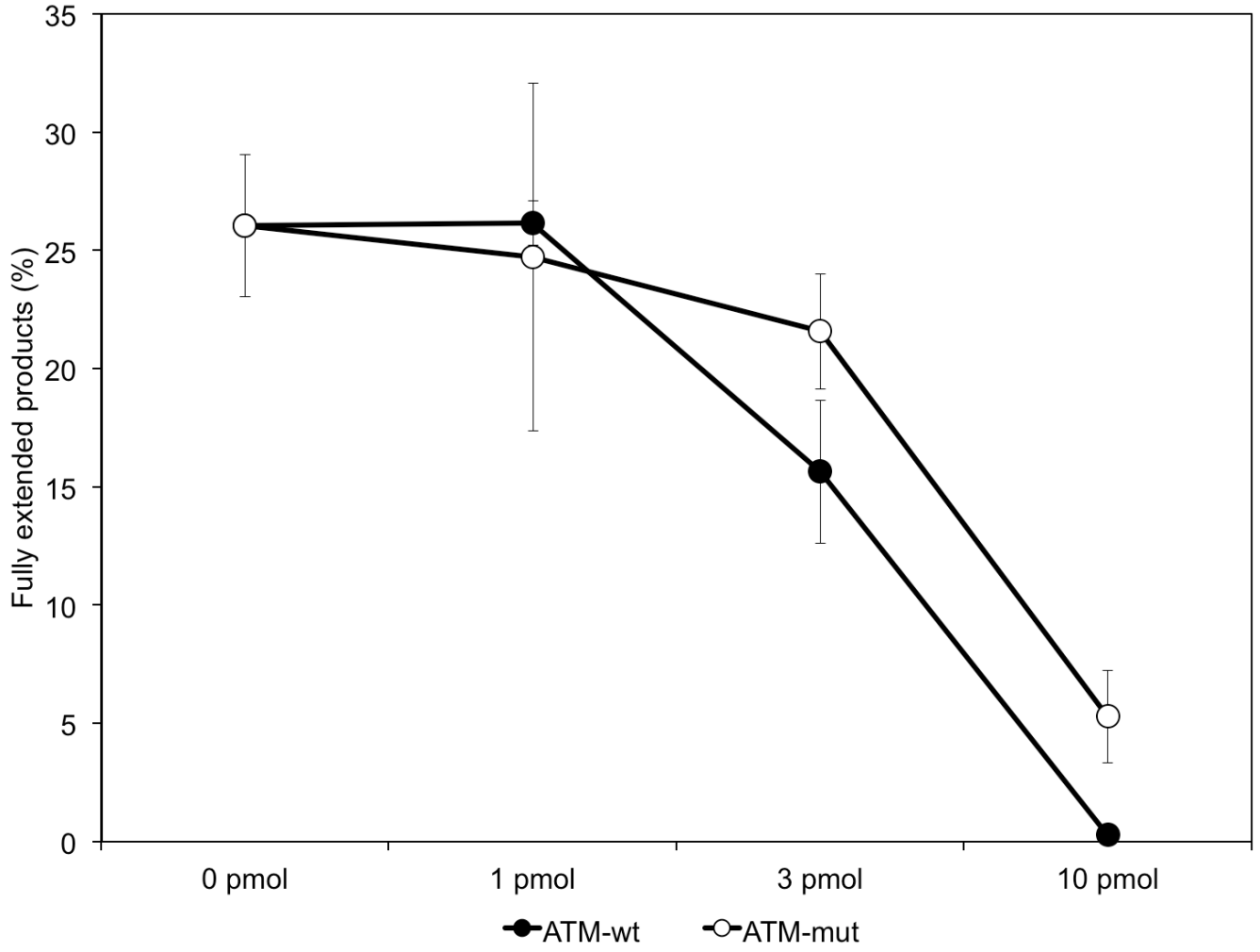

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Conclusions

Irreversible damage accumulates rapidly when ATM kinase activity is inhibited in irradiated cells

Chemically-inhibited ATM kinase blocks DNA repair in a manner that does not occur in the absence of ATM protein

ATM interacts with PCNA *in vivo* and *in vitro*

A 20 amino acid **ATM peptide stimulates DNA synthesis** by DNA polymerase δ *in vitro*

Funding

- R01CA148644
- 5P50CA090440 Lung SPORE
  - Developmental Project
  - Career Development Project
- 5U19AI068021
  - Developmental Project
- LCRF
  - Merit Award 2009, 2010
- Shapira Foundation
- BCRF

Acknowledgements

- Simon Watkins, PhD
  - Greg Gibson
- Alan Tomkinson, PhD
  - Yoshihiro Matsumoto, PhD
  - Dibyendu Banjerjee, PhD
- Richard Pelroy, PhD
- Arthur Levine, MD
- Joel Greenberger, MD
- Ben Van Houten, PhD
- Jill Siegfried, PhD
- Robert Sobol, PhD

Acknowledgements




Jason White, PhD	Serah Choi (MD, PhD. Student)	Armin Gamper, PhD gampera@upmc.edu	Katherine Fu
2006-2009	2007-2011	2010-present	2011-present